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COMPARATIVE PATHOLOGY OF INDUCED TUMORS OF THE SALIVARY GLANDS

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The experiments reported in this paper were originally designed to shed light on the causation and the essential histologic nature of the "mixed tumors of salivary gland type" seen in man. If such tumors could be induced in animals at will, their origin from "embryonal rests" would be extremely improbable, especially since such tumors rarely, if ever, develop in animals spontaneously.[1] Also their cytologic origin could be studied by putting animals to death at all stages of tumor development.

After it became apparent that "mixed tumors" of the human type could not be induced in rats with 3,4-benzpyrene, the experiments were extended to the use of other cancerogens, namely, 20-methylcholanthrene and 1,2,5,6-dibenzanthracene, and to other animals—mice, guinea pigs and rabbits—in the hope that among these the proper balance between cancerogen and host tissue might result in such tumors. Although tumors of the type originally sought were never produced, mixed tumors of other types were induced. These together with other features of interest form the basis of this report.

Spontaneous tumors of the "mixed salivary gland type" have not been reported in lower animals, although mixed tumors of other types have been described in various organs of several species.

Tumors induced in the salivary glands of animals have been reported. Steiner [2] described epidermoid cyst, squamous cell carcinoma and carcinosarcoma induced in rats and mice with 3,4-benzpyrene and methyl-

From the Department of Pathology of the University of Chicago.

1. Feldman, W. H.: Neoplasms of Domesticated Animals, Philadelphia, W. B. Saunders Company, 1932. Ratcliffe, H. L.: Am. J. Cancer **17**:116, 1933. In a personal communication to the author, Dr. J. S. Bengston, associate veterinarian in charge of the pathologic laboratory of the Bureau of Animal Industry, United States Department of Agriculture, Chicago, writes: "I fail to find any record of salivary gland tumors in food-producing animals in our laboratory records, which date back to 1906."

2. Steiner, P. E., in Program of the Third International Cancer Congress, Atlantic City, Sept. 11-16, 1939, p. 138.

cholanthrene. Benecke and Schröder [3] produced squamous cell carcinoma in 1 mouse and sarcoma of the parotid gland in mice and rats with benzpyrene. Rusch, Baumann and Maison [4] reported sarcoma and squamous cell carcinoma of the submaxillary gland induced in mice with benzpyrene and sarcoma induced in rats with dibenzanthracene. Franseen, Aub and Simpson [5] produced "abscesses" and squamous cell carcinoma in rats with methylcholanthrene, and epidermoid carcinoma and fibrosarcoma in mice. No mixed tumors have been reported.

Fig. 1.—Guinea pig showing a squamous cell carcinoma which arose in an epidermoid cyst.

EXPERIMENTAL STUDIES

In the experiments to be presented cylindric pellets were prepared according to the method of Shear.[6] Each pellet consisted of a carcinogenic hydrocarbon plus cholesterol, or of cholesterol alone, melted and drawn into a capillary glass tube. The solidified chemicals were removed and implanted in the left salivary gland by open surgical operation, the right gland serving as control. An attempt was made to place the pellet in the submaxillary salivary gland, but subsequent microscopic examination disclosed that the other salivary glands were sometimes in contact with the chemical, especially in the smaller animals.

3. Benecke, E., and Schröder, J.: Ztschr. f. Krebsforsch. **49**:505, 1939.

4. Rusch, H. P.; Baumann, C. A., and Maison, G. L.: Arch. Path. **29**:8, 1940.

5. Franseen, C. C.; Aub, J. C., and Simpson, C. L.: Cancer Research **1**:489, 1941.

6. Shear, M. J.: Am. J. Cancer **26**:322, 1936.

At the intervals of time indicated in the tables, the salivary glands were removed, and if no large tumor was present, they were flattened on a firm surface and fixed in this position. After paraffin embedding, subserial sections were cut. In this way the specific type of salivary gland, or other type of tissue, which was in relation to the pellet at various levels could be determined as well as the cytologic reactions to it. If a large tumor was present, it was removed together with portions of the surrounding tissues and fixed, after which several blocks of tissue were selected for microscopic study. When a large tumor was present, its exact site of origin, whether from a submaxillary, a sublingual or a parotid gland, could usually not be determined.

Extrusion of the pellet through the operative incision before healing took place was occasionally seen. Careful dissection at autopsy followed by microscopic

TABLE 1.—*Experiment 1. Methylcholanthrene in Mice*

Animal	Survival	Diagnosis
1 to 21	1-13 weeks	(See text)
22	3.5 months	Spindle cell sarcoma
23	3.75 months	Squamous cell carcinoma
24	4.75 months	Spindle cell sarcoma
25	4.75 months	Spindle cell sarcoma
26	6 months	Spindle cell and mixed cell sarcoma
27	6.25 months	Acanthoadenocarcinoma
28	6.25 months	Spindle cell sarcoma
29	6.75 months	No epithelial change
30	7 months	Mixed cell and spindle cell sarcoma
31	7 months	No epithelial change
32	7 months	Spindle cell sarcoma
33	8.25 months	Spindle cell sarcoma
34	8.5 months	Spindle cell sarcoma
35	8.5 months	Spindle cell sarcoma
36	9 months	Cavernous hemangiosarcoma
37	9.25 months	Squamous cell metaplasia
38	9.5 months	Acanthocarcinosarcoma
39	9.5 months	Spindle cell sarcoma
40	9.75 months	Spindle cell sarcoma
41	10.5 months	Acanthocarcinosarcoma
42	11.5 months	Spindle cell sarcoma
43	12 months	Squamous cell carcinoma Cavernous hemangiosareoma
44	12 months	Adenocarcinoma
45	13 months	Adenocarcinosarcoma
46	15 months	Adenocarcinosarcoma
47	17.25 months	Hemangiosarcoma

examination failed to disclose a pellet in a few animals in some of the experiments. Such animals are not included in the text or tables since this would not add to the value of the data but would indicate only the degree of skill of the operator. Several animals are excluded because microscopic examination showed that the pellets, although present, were not near the salivary glands.

In mice the main salivary glands—submaxillary, sublingual and parotid—are in close relationship to one another, lying mostly within the space enclosed by the rami and angles of the mandible and forming, together with lymph nodes, the bulging mass in the upper part of the neck which is so conspicuous in this species. In mice the submaxillary gland is generally considered to be a "modified serous," the sublingual a mucous and the parotid a serous gland (fig. 2*A*).[7]

The three main pairs of salivary glands in rats resemble grossly and microscopically those of mice except that they appear to be relatively smaller. How-

7. Fekete, E., in Snell, G. D.: Biology of the Laboratory Mouse, Philadelphia, The Blakiston Company, 1941.

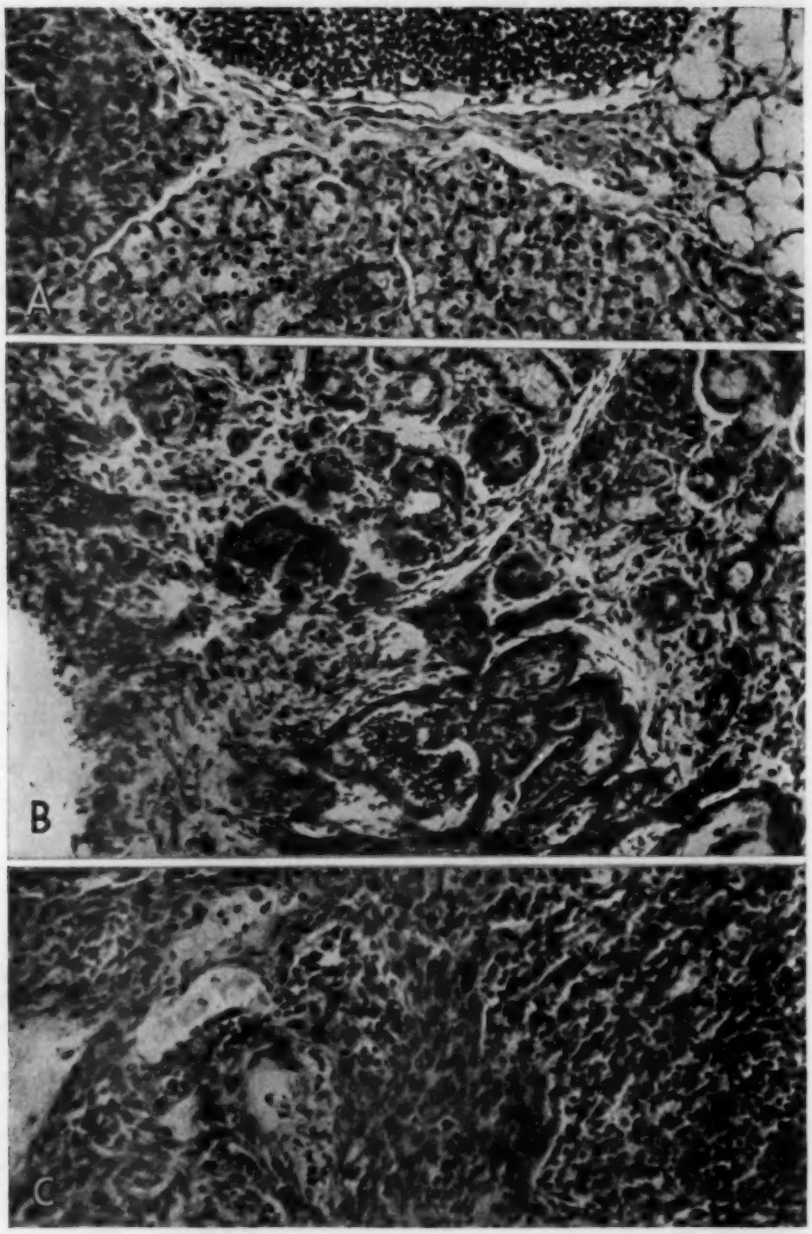

Fig. 2.—Photomicrographs of mouse tissue. *A* shows a parotid gland at the left, a submaxillary gland below, a sublingual gland at the right and a lymph node above (× 223). *B* shows epithelial metaplasia induced by methylcholanthrene in a submaxillary gland at fourteen days (× 162). The margin of the pellet space is at the lower left, and the submaxillary gland is at the upper right. *C* shows acanthocarcinosarcoma induced by methylcholanthrene in the submaxillary gland (× 273).

ever, because they are actually larger, it was easier to implant the pellet entirely within the submaxillary glands, despite the larger pellets used.

In guinea pigs the salivary glands are less discrete and less well encapsulated than in mice, rats and rabbits. Consequently those in obese adults show much fat infiltration. Furthermore, the glands are not sharply set apart from the thymus gland. For these reasons, and also because of the larger size of the pellets used, this experiment was less satisfactory than the others.

In rabbits, for anatomic reasons, the submaxillary gland was used exclusively. It is large and easily identifiable.

Experiment 1 (Methylcholanthrene in Mice).—Cylindric pellets consisting of 1 mg. of methylcholanthrene plus 1.5 mg. of cholesterol and measuring approximately 1 mm. in diameter and 2 mm. in length were implanted in the salivary glands of 46 mice of a partly inbred stock. They were from 44 to 74 days old at the time of operation and were about equally divided between the sexes.

Mice which grossly appeared to be without tumors were put to death at weekly intervals to study the early cytologic changes. Twenty-one were so examined between the first and thirteenth weeks inclusive. Of these, 6 showed only fibrous encapsulation of the pellet and no epithelial changes. The other 15 all showed epithelial metaplasia, glandular epithelium near the pellet having been converted into stratified squamous epithelium. Such metaplastic epithelium also lined the pellet space in 2 mice. In all instances it formed epithelial bodies, some of which had cystlike centers. The metaplasia occurred quickly, being present in both the mice examined at seven days. The transformation took place from both acinous and ductal epithelium. The former was especially well seen in instances in which the pellet was in contact only with the surface of the gland. Epithelial metaplasia occurred usually in the submaxillary gland and occasionally in the sublingual gland; it never occurred in the parotid gland, although the pellet was in relation to the latter in 4 mice. In 1 mouse the epithelial changes resembled beginning carcinoma.

Twenty-six mice lived longer than thirteen weeks. One of them showed only microscopic squamous metaplasia, 1 had carcinoma of the mammary gland arising in the neck, with no changes about the pellet located in the salivary gland, and 1 showed no epithelial changes in relation to the pellet. Twenty-three mice had twenty-four tumors, believed to have originated in the salivary glands. The types of tumors were: spindle and mixed cell sarcoma, thirteen tumors; squamous cell carcinoma, two; adenocarcinoma, one; adenocarcinosarcoma, two; acanthocarcinosarcoma, two; acanthoadenocarcinoma, one, and hemangiosarcoma, three.

One mouse had a tumor diagnosed as squamous cell carcinoma and a tumor diagnosed as hemangiosarcoma, both located in the salivary glands and colliding but otherwise appearing to be independent.

Experiment 2 (1,2,5,6-Dibenzanthracene in Mice).—Cylindric pellets consisting of 1 mg. of 1,2,5,6-dibenzanthracene and 1 mg. of cholesterol were implanted in the submaxillary glands of 20 male mice of the same stock as those used in experiment 1. They were 5 months old at the time of operation.

The mice died at the intervals shown in table 2. The early mortality was due almost entirely to infection of the genitourinary tract and to tumors. Six mice at autopsy showed no microscopic glandular changes, 4 showed epithelial metaplasia to a stratified squamous epithelium, and 10 showed neoplasms. Eight of these tumors were diagnosed as squamous cell carcinoma, 1 as acanthoadenocarcinoma and 1 as epidermoid cyst.

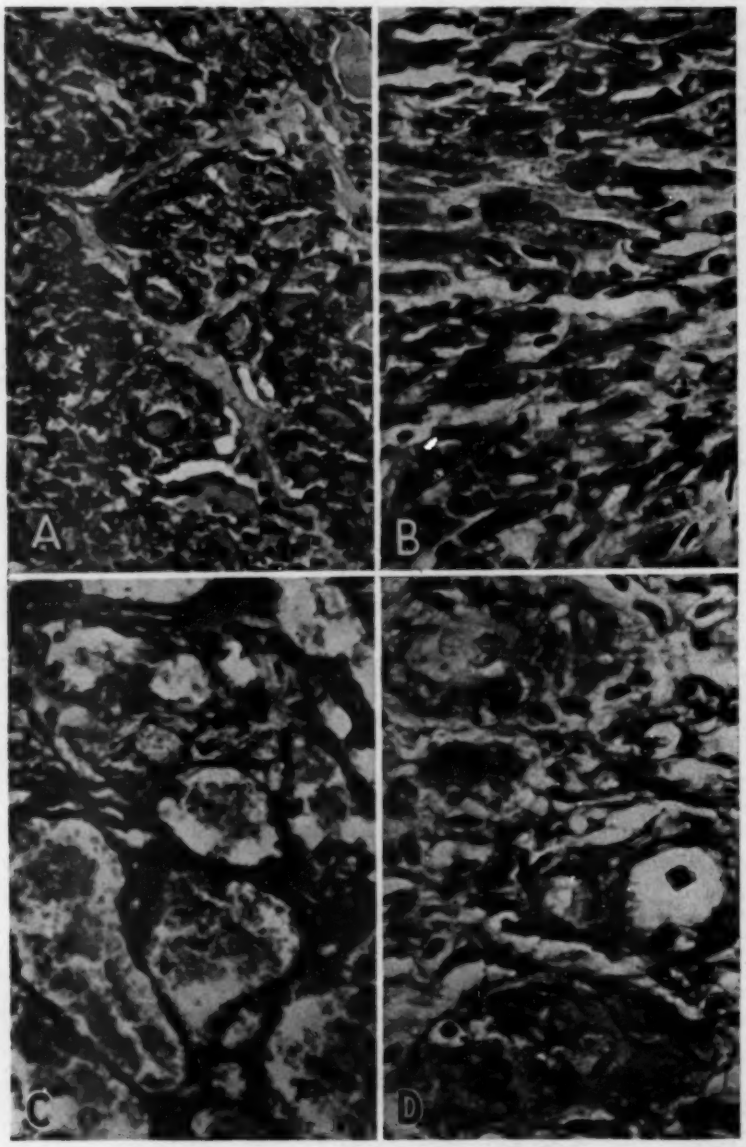

Fig. 3.—Photomicrographs. *A* shows adenocarcinoma arising from the submaxillary gland of a mouse in relation to a pellet of methylcholanthrene (× 275). *B* shows spindle cell and polymorphus cell sarcoma induced in the salivary gland of a mouse with methylcholanthrene (× 400). *C* shows angiosarcoma induced in the submaxillary gland of a mouse with methylcholanthrene (× 275). *D* shows adenocarcinosarcoma induced in a salivary gland of a rat with 3,4-benzpyrene (× 520).

Experiment 3 (Cholesterol in Mice).—Cylindric pellets consisting of 2.5 mg. of cholesterol were placed in the submaxillary glands of 21 mice of the same stock as that used in experiments 1 and 2. The mice were all females, and they were 60 to 64 days old at the time of operation.

Fifteen were killed at intervals of between one and twelve weeks for microscopic study. The other 6 lived over eight months, 5 lived over seventeen months and 2 lived over twenty-four months. No tumors of the salivary glands and no epithelial metaplasia were seen in this experiment. The reaction to the pellet consisted of a fibrous encapsulation together with a severe inflammatory reaction,

TABLE 2.—*Experiment 2. Dibenzanthracene in Mice*

Animal	Survival	Diagnosis
1	26 days	Squamous cell metaplasia
2	27 days	No epithelial changes
3	36 days	Squamous cell metaplasia
4	3 months	Squamous cell metaplasia
5	4 months	Squamous cell carcinoma
6	5 months	Squamous cell carcinoma
7	5.75 months	Acanthoadenocarcinoma
8	5.75 months	Squamous cell carcinoma
9	6 months	No epithelial changes
10	7 months	No epithelial changes
11	8 months	No epithelial changes
12	8 months	Squamous cell carcinoma
13	8 months	Large tumor—type ?
14	8.5 months	Squamous cell carcinoma
15	8.5 months	No epithelial changes
16	9.5 months	Epidermoid cyst
17	10 months	Squamous cell carcinoma
18	10.75 months	Squamous cell metaplasia
19	12.75 months	Squamous cell carcinoma
20	13.5 months	No epithelial changes

TABLE 3.—*3,4-Benzpyrene in Rats*

Animal	Survival	Diagnosis
1	13 months	Squamous cell carcinoma
2	16 months	Squamous cell carcinoma
3	16 months	Spindle cell and mixed cell sarcoma
4	16.5 months	Carcinosarcoma
5	16.5 months	Squamous cell carcinoma
6	17 months	No tumor
7	17.5 months	No tumor
8	17.75 months	Spindle cell and mixed cell sarcoma
9	18 months	No tumor

followed later by calcification of the pellet. The inflammatory reaction was definitely greater than that to cancerogenic chemicals seen in experiments 1 and 2.

Experiment 4 (Benzpyrene in Rats).—Cylindric pellets containing 3 mg. of 3,4-benzpyrene and 7 mg. of cholesterol were implanted in the salivary glands of 9 rats. The animals were litter mates, 50 days old, and the average weight of the 7 males was about 115 Gm. and of the 2 females about 90 Gm.

Squamous cell carcinoma (3 tumors), spindle and mixed cell sarcoma (2) and carcinosarcoma (1) were found at the periods of time shown in table 3. In 3 animals the pellet was present without tumor.

Experiment 5 (Methylcholanthrene in Rats).—Cylindric pellets consisting of 5 mg. of methylcholanthrene plus 5 mg. of cholesterol were implanted in the salivary glands of 16 rats. These were from a stock inbred by brother to sister mating for six generations. They were about 1 year old at the time of operation.

The animals survived to the periods of time shown in table 4, death having been due to chronic and acute pneumonia in nearly every case. In 1 rat spindle cell sarcoma developed; 2 others died without epithelial changes, although examination disclosed the pellets lying in their submaxillary gland. All other animals

TABLE 4.—*Methylcholanthrene in Rats*

Animal	Survival	Diagnosis
1	12 days	Squamous cell metaplasia
2	1.5 months	Squamous cell lining of pellet space and glandular cell metaplasia
3	1.5 months	No epithelial changes
4	1.75 months	Squamous cell lining of pellet space
5	2.5 months	Squamous cell lining of pellet space
6	2.5 months	Squamous cell lining of pellet space and small squamous cell cysts
7	3 months	Squamous cell lining of pellet space and glandular cell metaplasia
8	4.75 months	Squamous cell lining of pellet space and glandular cell metaplasia
9	5.25 months	Squamous cell lining of pellet space
10	7 months	Squamous cell carcinoma
11	8.5 months	Epidermoid cyst
12	9 months	Spindle cell sarcoma
13	9.75 months	Epidermoid cyst with squamous cell carcinoma
14	11 months	Epidermoid cyst with squamous cell carcinoma
15	11.5 months	Squamous cell carcinoma
16	12.25 months	No epithelial changes

TABLE 5.—*Methylcholanthrene in Guinea Pigs*

Animal	Survival	Diagnosis
1	7 days	No epithelial change
2	7 days	No epithelial change
3	7 days	No epithelial change
4	14 days	No epithelial change
5	14 days	No epithelial change
6	24 days	No epithelial change
7	31 days	Epithelial lining of pellet space and adjoining squamous cell metaplasia
8	32 days	Epithelial lining of pellet space
9	3 months	Epithelial lining of pellet space
10	4.5 months	No epithelial change
11	8.5 months	No epithelial change
12	11 months	Epidermoid cyst
13	12 months	Squamous cell metaplasia near pellet
14	13 months	No epithelial change
15	13 months	Epidermoid cyst
16	13.5 months	Epidermoid cyst
17	15.5 months	Epidermoid cyst
18	16.25 months	Spindle cell sarcoma
19	22 months	Epidermoid cyst with carcinomatous features
20	22.5 months	No epithelial changes
21	22.5 months	Squamous cell carcinoma
22	24.5 months	Epidermoid cyst
23	25.75 months	Epidermoid cyst
24	25.75 months	Squamous cell carcinoma
25	26 months	Squamous cell carcinoma

showed epithelial changes. These took the form of squamous metaplasia or of epithelization of the pellet space or both in animals dying during the first six months and of epidermoid cysts with or without squamous cell carcinoma arising in the wall, or noncystic squamous cell carcinoma, in animals dying thereafter.

Experiment 6 (Methylcholanthrene in Guinea Pigs).—Cylindric pellets containing 10 mg. of methylcholanthrene plus 10 mg. of cholesterol and measuring about 1.75 mm. in diameter and 8 to 10 mm. long were implanted into the salivary glands of 25 guinea pigs. They were young, weighing from 400 to 500 Gm.

Despite the large amount of cancerogen used, the first epithelial metaplasia and epithelization of pellet space were seen at thirty-one days. The earliest evidence for progressive epithelial growth was seen at eleven months in the form of an epidermoid cyst. Additional cysts were subsequently seen but epithelial neoplastic conversion was not seen until twenty-two and one-half months. Spindle cell sarcoma was encountered at sixteen and one-half months.

Experiment 7 (Cholesterol in Guinea Pigs).—Pellets consisting of 20 mg. of cholesterol were implanted into the salivary glands of 3 young guinea pigs. They were killed at fourteen days (1 guinea pig) and at twenty-eight days. Microscopic examination disclosed no epithelial change in relation to the pellets.

Experiment 8 (Methylcholanthrene in Rabbits).—Eleven young rabbits were used. Each received 10 mg. of methylcholanthrene in 1 cc. of sesame oil into the left submaxillary gland. The gland was exposed surgically and the injection made under direct vision. Back leakage through the needle tract was seen in about half the animals.

The animals survived for periods varying from twenty-four hours to twenty-three months, as indicated in table 6. The epithelial changes seen at postmortem

TABLE 6.—*Methylcholanthrene in Rabbits*

Rabbit	Survival	Epithelial Changes
1	1 day	None
2	3 days	Epithelial metaplasia
3	22 days	None
4	3 months	Epithelial metaplasia and squamous cysts
5	4 months	Epithelial metaplasia
6	10 months	Epithelial cyst
7	17 months	None
8	20 months	Epithelial metaplasia
9	23 months	None
10	23 months	None
11	23 months	None

examination consisted of epithelial metaplasia and epithelial cyst formation. The connective tissue changes were those of granuloma formation and fibrous tissue increase in proximity to the chemical. Epithelial metaplasia near the chemical was seen as early as the third day. Later some of the squamous epithelial bodies developed into small epidermoid cysts. The largest epithelial cyst encountered was 3.5 mm. in diameter. It was lined by low columnar cells. The cysts did not appear to grow progressively. No malignant tumors were produced in this experiment.

The speed of metaplasia of glandular to stratified squamous epithelium (three days) was in marked contrast with the subsequent behavior, namely, the failure of conversion to malignant neoplasia. This might indicate resistance of this species to such change; on the other hand, it may merely be the result of inadequate exposure to the cancerogen. Pellets of cancerogen should be used before the latter possibility is excluded.

COMMENT

The results in experiments 1 to 8 are summarized in tables 7 and 8. As used here, "induction time" refers to the time of death of the tumor-bearing animal and not to the time of first appearance of the tumor. The "effective total" is the number of animals living in the experiment when the first tumor-bearing animal died.

In table 7 is listed the wide variety of tumors which arose in the salivary glands. The tumors of connective tissue were diagnosed as spindle and mixed cell sarcoma. Presumably they arose from the stroma

TABLE 7.—*Summary of Experiments in Which Epithelial Metaplasia and Tumors Were Observed*

			Tumors of Given Type Produced										
Experiment	Species	Chemical	No Epithelial Change	Metaplasia to Squamous Cells	Epidermoid Cyst	Sarcoma	Hemangiosarcoma	Adenocarcinosarcoma	Acanthocarcinosarcoma	Acanthoadenocarcinoma	Adenocarcinoma	Squamous Cell Carcinoma	Total
1	Mouse	Methylcholanthrene	8	16	..	13	3	2	2	1	1	2	47*
2	Mouse	1,2,5,6-dibenzanthracene	6	4	1	..	..	..	..	1	..	8	20
3	Mouse	Cholesterol	21	..	..	..	..	..	..	..	..	..	21
4	Rat	3,4 benzpyrene	3	..	..	2	..	1	..	..	..	3	9
5	Rat	Methylcholanthrene	2	8	1	1	..	..	..	..	..	4	16
6	Guinea pig	Methylcholanthrene	10	4	7	1	..	..	..	..	..	3	25
7	Guinea pig	Cholesterol	3	..	..	..	..	..	..	..	..	..	3
8	Rabbit	Methylcholanthrene	6	4	1	..	..	..	..	..	..	..	11

* One mouse had two tumors.

TABLE 8.—*Summary of Experiments in Which Carcinomatous Changes Were Induced*

Experiment	Species	Chemical	Amount of Chemical, Mg.	Induction Time for First Tumor, Mo.	Effective Total of Animals	Tumors	Percentage Yield of Tumors	Average Induction Time, Mo.
1	Mouse	Methylcholanthrene	1	3.75	11	8	72.7	10.25
2	Mouse	1,2,5,6-dibenzanthracene	1	4	15	10	66.6	8.2
3	Mouse	Cholesterol	2.5	..	..	0		
4	Rat	3,4 benzpyrene	3	13	6	3	50.0	15.3
5	Rat	Methylcholanthrene	5	7	6	5	83.3	9.5
6	Guinea pig	Methylcholanthrene	10	11	5	3	60.0	24.75
7	Guinea pig	Cholesterol	20	..	..	0		
8	Rabbit	Methylcholanthrene	10	..	..	0		

within or around the glands. They showed no differences from sarcoma induced in other tissues in their appearance, induction time, rate of growth or other features. Attempts at serial transplantation were carried out with two of these tumors which arose in mice. Both were successful.

The tumors here called cavernous hemangiosarcoma are of interest because they have their counterpart in human salivary glands,[8] because of their long induction time (average, twelve and eight-tenths months) and because they are rarely induced in other tissues, although they have been reported in the liver by Furth.[9] They were recognizable during life by their blue-black color.

The malignant monotypic epithelial tumors consisted of squamous cell carcinoma and adenocarcinoma (1 instance). They showed no unusual morphologic features except that some of them clearly arose from the squamous cell lining of epidermoid cysts.

The earliest changes in epithelium are of greater interest because the resultant cells were already abnormal. They were irregular in size, shape, staining qualities and in relation to each other, failing to form orderly sheets of stratified squamous epithelium. The nuclei were hyperchromatic and the cytoplasm more acidophilic than normal. Later, in some of the epidermoid cysts, particularly in guinea pigs, and in layers of epithelium lining pellet spaces, fully benign-appearing, orderly growth was seen. Spontaneous metaplasia due to vitamin deficiency was not seen in control glands.

It appears that the glandular epithelium of the salivary glands shares with some other glandular epitheliums, notably prostatic,[10] the tendency to undergo metaplasia easier than neoplasia, in response to the same chemical agent, but that the continued action of the cancerogen may eventually produce a tumor. This raises the interesting question as to whether metaplasia is of the same order as neoplasia or whether they are entirely different but sometimes associated processes. The former sometimes precedes the latter in tumors of the human lung, the urinary bladder, the renal pelvis and the gallbladder. The important question as to whether the cells observed were truly neoplastic with a capacity for unlimited and independent growth from the time of the earliest morphologic change must remain unanswered since no tests for transplantability were made at that stage.

The tumors which showed several types of cells are of special interest. They occurred mostly in mice and under the influence of methylcholanthrene. The question arises as to whether they were true mixed tumors or whether they were combined or "collision" tumors. Some of them were clearly of the latter type. For example, 1 mouse had two tumors, squamous cell carcinoma and hemangiosarcoma, both growing in the submaxillary gland. Morphologic evidence for true mixed tumors was seen in other cases, where transition forms of cells between well differentiated forms of carcinoma and of sarcoma were seen.

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The tables show an interesting peculiarity which may be attributed to the cancerogens. In experiments 1 and 2 the stock of mice was the same, as was the amount of cancerogen, yet methylcholanthrene induced sarcoma in many mice, mixed tumors in numerous ones, hemangiosarcoma in some and squamous cell carcinoma in a few, while dibenzanthracene produced the last almost exclusively. In rats methylcholanthrene induced tumors in a higher percentage with a shorter induction time than did 3,4-benzpyrene, but this difference might be explained on the basis of different dosage.

Differences which may be attributed to the species are also seen in the tables.

SUMMARY

Under the influence of the cancerogenic hydrocarbons methylcholanthrene, 1,2,5,6-dibenzanthracene and 3,4-benzpyrene, a wide variety of cell changes, ranging from metaplasia to neoplasia, were induced in the salivary glands of mice, rats, guinea pigs and rabbits. Metaplasia of the acinous and terminal duct cells from a glandular or cuboidal to a squamous type of epithelium occurred quickly, being seen as early as the third day. Such metaplastic cells were already abnormal, and the question of the relationship of metaplasia to neoplasia is again raised.

The types of epithelial neoplasm seen later were epidermoid cyst, squamous cell carcinoma and adenocarcinoma. The types of connective tissue neoplasm were spindle cell and mixed cell sarcoma. The mixed tumor types seen were adenocarcinosarcoma, acanthocarcinosarcoma and acanthoadenocarcinoma, but none composed of epithelium and cartilage. Three blood vessel tumors, called hemangiosarcoma, were also seen. From the observations on mice it appears that the submaxillary salivary gland is more susceptible to neoplastic conversion than the sublingual or the parotid gland.

Epithelial tumors were produced in rats and guinea pigs, in which the induction of epithelial tumors is generally considered to be difficult. The wide variety of types of tumors seen is of special interest in view of the fact that the commonest variety seen in man, the mixed tumor containing epithelium and cartilage, did not occur. The ease of the induction of tumors in the salivary glands is in marked contrast with the rarity of such spontaneous tumors in animals. Methylcholanthrene produced more sarcoma and also a greater variety of mixed tumors in mice than did dibenzanthracene. In rabbits squamous metaplasia was produced but not neoplasia.

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MECHANISM OF PARATHYROID HORMONE ACTION

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In 1929 I [1] reported on "calcium gout" or metastatic calcification in a patient who had a large parathyroid tumor (adenoma). At this time I considered the possibility that the calcium depositions in soft tissues were the result of an overproduction of the parathyroid hormone but emphasized that the observations did not definitely prove this.

Shortly afterward Bodansky and co-workers [2] and Jaffe and co-workers [3] published their classic observations concerning the production of osteitis fibrosa with an extract containing the parathyroid hormone, and Jaffe and others [4] came to the conclusion that these bone lesions "may come about as the result of a change in the circulating tissue fluid, the salts being dissolved out of the organic matrix and the latter disappearing secondarily." My experimental observations in rats treated with large doses of a parathyroid extract [5] led me to believe that the primary site of parathyroid hormone action is in the bone itself. In osseous tissue, acute overdosage with comparatively large doses of this extract stimulates osteoclast formation and bone absorption, and this is often accomplished by fibrosis in those areas in which the weakened bones are exposed to mechanical strain. In the case of chronic treatment with moderate doses, the hormone stimulates the formation of osteoblasts and the deposition of new bone tissue so that a condition simulating the marble bone disease of Albers-Schönberg results. On the basis of these and similar observations, Thomson and Collip,[6] who carefully reviewed the relevant literature until 1932, likewise expressed themselves in favor of the foregoing interpretation.

However, others [7] advanced and still defend the theory that the primary point of attack of the parathyroid hormone is in the kidney

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since the first result of the administration of a parathyroid extract which they could detect was a lowering of the renal threshold for phosphorus accompanied by an increased elimination of phosphate and a decrease in the inorganic phosphorus content of the serum. Similar observations induced a number of subsequent investigators to accept the renal theory of parathyroid hormone action.[8] Tweedy and co-workers [9a] claimed that "Selye's theory is necessarily based upon the assumption that the blood is undersaturated with respect to calcium." This is not necessarily so. The literature concerning the solubility of calcium in normal blood is rather contradictory since this is influenced by a great many chemical and physical factors. It is evident, however, that whatever the primary site of parathyroid hormone action may be, the resulting deposition of calcium in the various soft tissues proves that the calcium is held in the blood in a rather labile condition reminiscent of oversaturated solutions. Tweedy and co-workers [9] claimed that the increase in the phosphate content of the serum which follows bilateral nephrectomy in the dog cannot be prevented by the administration of an extract containing the parathyroid hormone. Furthermore, during renal insufficiency such an extract fails to cause any significant rise in blood calcium and nephrectomized dogs are protected from parathyroid overdosage effects. This confirmed the belief of the authors that the mobilization of bone calcium into the blood by the parathyroid hormone is dependent on renal function. Recently similar experiments led even Collip [10] to abandon our view.

Although these experiments appeared to speak conclusively in favor of the renal theory, it must be kept in mind that in most abnormal conditions there is an inverse proportion between the calcium and the phosphate content of the blood and that consequently the failure to obtain more pronounced blood chemical changes after nephrectomy might have been due to the well known blood phosphate–raising effect of this intervention. I felt, therefore, that the crucial experiment which could decide between the renal and the osseous theory of parathyroid hormone action would be one to determine whether the typical changes in the bone could be produced after bilateral nephrectomy. This proved to be the case since Collip, Pugsley, Thomson and I [11] were able to elicit in completely nephrectomized rats marked osteoclastic bone resorption by treatment with a parathyroid extract. McJunkin and

8. Jones, J. H.: J. Biol. Chem. **115**:371, 1936. Morgan, A. F., and Samisch, Z.: ibid. **108**:741, 1935.

9. Tweedy, W. R.; Templeton, R. D., and McJunkin, F. A.: (*a*) Am. J. Physiol. **115**:514, 1936; (*b*) Endocrinology **21**:55, 1937.

10. Neufeld, A. H., and Collip, J. B.: Canad. M. A. J. **46**:188, 1942; Endocrinology **30**:135, 1940.

11. Collip, J. B.; Pugsley, L. I.; Selye, H., and Thomson, D. L.: Brit. J. Exper. Path. **15**:335, 1934.

co-workers,[12] who repeated these experiments, confirmed that an extract containing the parathyroid hormone causes osteoclastic resorption of bone, but since this was unaccompanied by any significant rise in blood calcium, they concluded that apparently "the calcium is removed from the bone into the extravascular soft tissues where it is held or that it is excreted by the intestines." The absence of hypercalcemia might have been due to the rise in blood phosphates occasioned by nephrectomy. However, both Tweedy and co-workers [13] and Ellsworth and Futcher [14] subsequently succeeded in producing definite hypercalcemia in nephrectomized dogs by administration of a parathyroid extract so that the renal theory of the action of the hormone would appear to be definitely disproved. Goadby and Stacey [15] found that in patients suffering from renal insufficiency a parathyroid extract fails to elicit the usual excretion of phosphate but effectively raises the blood calcium. They interpreted this observation as proving that at least in the normal person the parathyroid hormone acts directly on the kidney, lowering its phosphate threshold. Nonprotein nitrogen determinations following injections of parathyroid extracts are rather contradictory,[16] and it appears unlikely that the hormone of the parathyroids influences the nitrogen-eliminating power of the kidney in any significant manner.

Although I am not inclined to accept the theory postulating that the parathyroid hormone acts primarily on the kidney, it appears most probable that the kidney can secondarily influence the parathyroids. In my previously mentioned clinical case of metastatic calcification the parathyroid adenoma developed in a patient who suffered from severe renal damage,[1] and since that time several other similar cases have become known. Indeed, Highman and Hamilton [17] claimed to have demonstrated the presence of excess parathyroid hormone in the blood of patients suffering from chronic renal disease, and Pappenheimer [18] emphasized that the parathyroids enlarge in the rat after partial nephrectomy.

In the present communication I wish to present further experimental data indicating that the parathyroid hormone produces osteitis fibrosa even in the nephrectomized animal. It became evident, however,

12. McJunkin, F. A.; Tweedy, W. R., and McNamara, E. W.: Am. J. Path. **13**:325, 1937.

13. Tweedy, W. R.; Templeton, R. D., and McJunkin, F. A.: J. Biol. Chem. **109**:P92, 1935.

14. Ellsworth, R., and Futcher, P. H.: Bull. Johns Hopkins Hosp. **57**:91, 1935.

15. Goadby, H. K., and Stacey, R. S.: Biochem. J. **28**:2092, 1934; **30**:269, 1936.

16. Collip, J. B.; Clark, E. P., and Scott, J. W.: J. Biol. Chem. **63**:439, 1925. Remond, A.; Colombies, H., and Bernardbeig, J.: Compt. rend. Soc. de biol. **91**: 445, 1924. Berencsy, C. V.: Klin. Wchnschr. **9**:1213, 1930. Hajos, K., and Mazgon, R.: Ztschr. f. exper. Med. **70**:459, 1930.

17. Highman, W. J., Jr., and Hamilton, B.: J. Clin. Investigation **16**:103, 1937.

18. Pappenheimer, A. M.: J. Exper. Med. **64**:965, 1936.

that complete nephrectomy in itself may cause bone lesions similar to those produced by injection of a parathyroid extract. However, these lesions develop slowly, and if large doses of the extract are administered immediately after nephrectomy, it can be shown that the contained hormone acts before changes due to nephrectomy become marked. It was found, furthermore, that the bone changes caused by nephrectomy are prevented by simultaneous parathyroidectomy. This indicates that the kidney influences the bones by way of the parathyroids.

Another part of these investigations was concerned with the influence of partial hepatectomy and of thyroidectomy on the effectiveness of the parathyroid treatment. This appeared to be of importance since it was found that many hormones are detoxified in the liver [19] and that in animals receiving large doses of a parathyroid extract there is increased excretion of calcium through the bile,[20] and the liver shows morphologic signs of damage.[21] The importance of the thyroid has been emphasized by Régnier and Simonnet [22] and Riddle and Dotti,[23] who claimed that a given dose of a parathyroid extract causes a more pronounced rise of blood calcium in thyroidectomized than in intact animals.

METHODS

Inbred young albino rats were used for all the experiments. Nephrectomy and thyroidectomy were performed with the animals under ether anesthesia, the latter with the help of a binocular dissecting microscope. Partial hepatectomy was executed according to the technic described by Waelsch and Selye,[24] and it should be emphasized that the amount of liver tissue removed in this manner causes a sufficient degree of hepatic insufficiency to make possible the detection of drugs detoxified by liver cells. This has been shown for tribromethanol [24] and for a number of steroid hormones,[19] all of which proved distinctly more toxic to mice and rats partially hepatectomized in this manner than to intact controls. On the other hand, drugs not detoxified in the liver—e. g., magnesium chloride, ether, chloroform—are no more damaging to the partially hepatectomized than to the intact animal so that the increase in the sensitivity to certain drugs cannot be ascribed merely to a general decrease in resistance following this intervention.

The soft tissues were histologically examined for calcium deposits with the aid of von Kossa's method, and the bones were sectioned after decalcification in dilute nitric acid solution.

The blood nonprotein nitrogen was determined by the Folin-Wu method modified for microdetermination with the photoelectric colorimeter. It will be noted that the nonprotein nitrogen values obtained with the micromethod are somewhat higher than those obtained with the macromethods. This is merely due to the different

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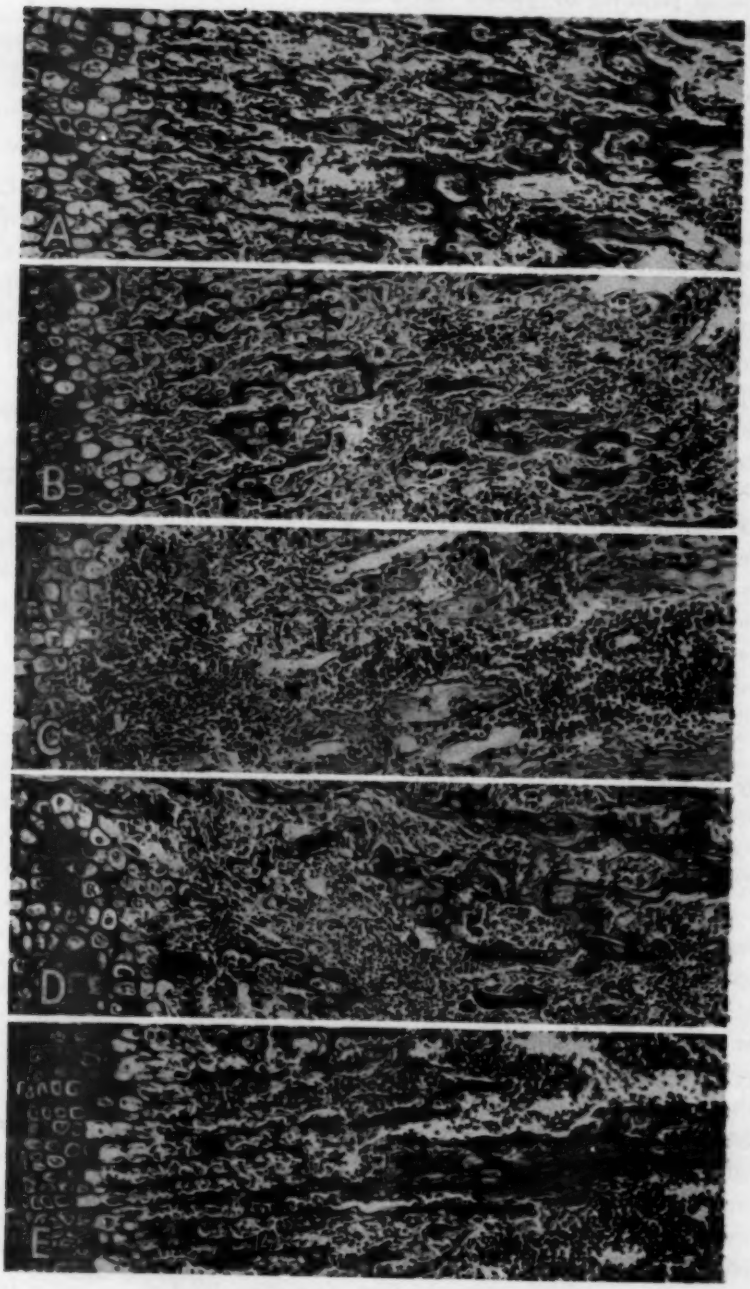
19. Selye, H.: J. Pharmacol. & Exper. Therap. **71**:236, 1941.
20. Kim, M. H.: Acta med. Keijo **12**:203, 1929.
21. Cantarow, A.; Stewart, H. L., and Housel, E. L.: Endocrinology **22**:13, 1938. Jaffe and others.[4]
22. Régnier, M. T., and Simonnet, H.: J. de pharm. et chim. **17**:560, 1933.
23. Riddle, O., and Dotti, L. B.: Proc. Soc. Exper. Biol. & Med. **32**:507, 1934.
24. Waelsch, H., and Selye, H.: Arch. f. exper. Path. u. Pharmakol. **161**:115, 1931.

technic of preparing the protein filtrate and does not interfere with comparative determinations.

The parathyroid extract used was made by Eli Lilly & Company and contained 20 U. S. P. parathyroid units per cubic centimeter. This preparation was supplied by Dr. H. W. Rhodehamel of that company.

EXPERIMENTAL STUDIES

In the first experimental series 48 rats weighing 49 to 70 Gm. (average, 57 Gm.) were used. These were divided into four equal groups, each consisting of 6 males and 6 females. Group 1 was thyroidectomized; group 2, bilaterally nephrectomized, and group 3, partially hepatectomized, according to the methods described in the previous section. Group 4 acted as intact controls. Sixteen hours after the surgical interventions were performed in the first three groups, the animals of all four groups received a 20 unit dose of parathyroid extract subcutaneously. A second dose of the same magnitude was injected two hours and a third four hours after the first dose. Thus each animal in each group received a total dose of 60 units. Twenty hours after the initiation of the treatment there were no casualties in any of the groups except the nephrectomized animals, among which 3 females and 3 males had died, and all the survivors were obviously weak, some of them dying. At this time, i.e., twenty hours after the first injection of the parathyroid extract, all the survivors were killed, and the kidneys, heart and femurs of each were histologically examined. It was found that metastatic calcification was not marked as yet in any of the soft tissues, although the lumens of the renal tubules contained deposits taking the von Kossa stain in all cases. It is of interest, therefore, that a study of the bones revealed an advanced degree of osteoclastic absorption of bone even at this early stage. This observation gives further support to the theory that the hormone of the parathyroids acts primarily on bone and causes calcification of soft tissue only secondarily, i.e., at a time when the kidney is unable to excrete all the calcium and phosphate liberated from the bones. The fact that both the degree of osteitis fibrosa and the number of von Kossa–stained cylinders in the renal tubules were practically the same in all four groups indicates that the thyroid, the kidney and the liver are not necessary for the action of the parathyroid hormone. The observation that many nephrectomized animals died and that all showed clinical symptoms of damage is not significant, however, since in this series there were no nephrectomized but otherwise untreated controls. For this reason a second experiment was performed on four groups of 6 female rats weighing 54 to 65 Gm. (average, 60 Gm.). Since in the previous experiment there appeared to be no difference in the responsiveness of males and females to the parathyroid hormone, it was thought permissible to perform this second experiment on animals of one sex



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only. Group 1 acted as untreated controls; group 2 were intact animals treated with the parathyroid extract; group 3 was nephrectomized but otherwise not treated, while the remaining 6 rats of group 4 were nephrectomized and treated with the parathyroid extract. In groups 2 and 4 a 60 unit dose was administered subcutaneously in three fractions at six hour intervals, the first injection being given immediately after the operation. Twenty hours after the nephrectomy the untreated control animals showed no obvious signs of uremia and appeared to be in fit condition, while the nephrectomized animals given the parathyroid extract were weak and showed marked tremor with occasional convulsions. Since some of the animals in the latter group were obviously dying at this time, we decided to put all the animals to death in order to obtain their blood for nonprotein nitrogen determinations. The blood nonprotein nitrogen in the intact controls ranged between 55 and 62 mg. per hundred cubic centimeters (average, 60 mg.); that in the intact animals given injections of the parathyroid extract, between 54 and 67 mg. (average, 59 mg.). In the nephrectomized but not treated group the corresponding values were 146 to 232 mg. (average, 204 mg.), while in the treated nephrectomized group the values ranged between 212 and 323 mg. (average, 222 mg.). Unfortunately, histologic studies were not made in this series.

These experiments clearly indicate that the parathyroid hormone does not lose its toxic properties after nephrectomy; on the contrary, the hormone proves more toxic in the absence than in the presence of kidney tissue. The experiments furnish no evidence that the thyroid plays an important part in the mechanism of parathyroid hormone action, and they show that at least no important proportion of the active factor in the injected extract is detoxified by the liver.

The observations mentioned in the introductory part of this paper raised the question whether nephrectomy might not itself cause osteitis fibrosa by stimulating the parathyroids. For this reason a third experiment was performed on five groups, each consisting of 6 male rats

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EXPLANATION OF FIGURE

A, femur of a normal rat. This and all subsequently shown sections are taken from the region just proximal to the distal junction of cartilage.

B, femur of a nephrectomized, otherwise untreated rat. Note the slight proliferation of fibrous tissue and the beginning resorption of trabeculae.

C, femur of a nephrectomized rat treated with a parathyroid extract, showing pronounced fibrous tissue formation with marked bone resorption.

D, femur of an intact rat treated with a parathyroid extract, showing changes similar to those seen in *C*.

E, femur of a nephrectomized and parathyroidectomized rat exhibiting a practically normal structure.

weighing 105 to 123 Gm. (average, 112 Gm.). Group 1 was merely nephrectomized and not otherwise treated; group 2 was nephrectomized and simultaneously parathyroidectomized; group 3 was nephrectomized and given subcutaneously a 100 unit total dose of the parathyroid extract in five fractions over a period of twenty-seven hours; group 4 remained intact but received the same amount of parathyroid extract as group 3. Group 5 consisted of intact, not treated normal animals. This smaller dose of the parathyroid extract did not suffice to kill any of these large rats, but its toxicity was evidently greater in the nephrectomized than in the normal group as judged by the general condition of the animals. Thirty-two hours after the initiation of treatment all animals were killed and their femurs histologically examined. As shown by the accompanying figure, the parathyroid extract caused pronounced osteoclastic bone absorption both in the intact and in the nephrectomized rat, while the slight osteitis fibrosa caused by nephrectomy itself was completely prevented by simultaneous parathyroidectomy.

On the basis of these experiments it appears justified to conclude that the parathyroid hormone does not act through the kidney, although renal function evidently is of importance for the readjustment to the metabolic disturbances caused by excess amounts of the hormone. Conversely, the kidney may secondarily influence the parathyroids and in this manner cause bone changes similar to those elicited by overdosage of solution of parathyroid and preventable by parathyroidectomy. This action of nephrectomy on the bones of the rat is well established by the experiments described in the foregoing paragraphs, although McJunkin and co-workers [12] claimed that even forty-eight hours after ablation of both kidneys the rat bones are histologically normal .

SUMMARY

Experiments on the rat indicate that complete nephrectomy does not prevent the action of the parathyroid hormone on bones. Nephrectomy itself elicits bone changes similar to those caused by the parathyroid hormone, though they are comparatively mild and develop more slowly. Parathyroidectomy prevents the bone changes caused by nephrectomy. Hence it is concluded that the hormone of the parathyroids does not act on the bones through the intermediary of the kidney as had been postulated by others. Nephrectomy, on the other hand, probably affects the bones because it stimulates the production of this hormone by the parathyroids.

Judged by the effect of administration of a parathyroid extract on partially hepatectomized or thyroidectomized rats, neither the liver nor the thyroid appears to influence the action of the parathyroid hormone significantly.

RENAL CHANGES IN THE ALBINO RAT ON LOW CHOLINE AND CHOLINE-DEFICIENT DIETS

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ST. LOUIS

In young rats a diet deficient in choline produces a pathologic state characterized by deposition of lipids in the liver, involution of the thymus, ocular hemorrhage, and congestion and enlargement of the kidneys (Griffith and Wade [1]). These manifestations of choline deficiency are aggravated by supplements of cystine and cholesterol and are prevented by supplements of choline, methionine and betaine (Griffith and Wade [2]; Griffith [3]). The relation of these substances to the occurrence and the prevention of fatty infiltration of the liver in older rats has been reviewed by Best and Ridout.[4] Du Vigneaud and co-workers [5] have demonstrated transmethylation in the rat and have suggested the utilization of the methyl groups of methionine and of betaine in the synthesis of choline. The observation that organs, especially the kidneys, undergo marked degeneration in young animals fed a diet which is low in choline and adequate in protein indicates that young rats require greater amounts of choline than older ones and that the highly purified diets commonly employed in nutritional studies may be deficient both in choline and in such substitutes for choline as methionine and betaine. Renal lesions of dietary origin in rats have been reported by several investigators, who have attributed them either to supplements of cystine or to a deficiency in the vitamin B complex (Hartwell [6]; Cox and Hudson [7]). The effect of cystine on the kidneys has not been

From the Department of Microanatomy, St. Louis University School of Medicine.

1. Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **131**:567, 1939.
2. Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **132**:627, 1940.
3. Griffith, W. H.: (*a*) J. Biol. Chem. **132**:639, 1940; (*b*) J. Nutrition **21**: 291, 1941.
4. Best, C. H., and Ridout, J. H., in Luck, J. M., and Smith, J. H. C.: Annual Review of Biochemistry, Stanford University, Calif., Annual Reviews, Inc., 1939, vol. 9, p. 349.
5. du Vigneaud, V.; Chandler, J. P.; Moyer, A. W., and Keppel, D. M.: J. Biol. Chem. **131**:57, 1939. du Vigneaud, V.; Chandler, J. P.; Cohn, M., and Brown, G. B.: ibid. **134**:787, 1940.
6. Hartwell, G. A.: Biochem. J. **22**:1212, 1928.
7. Cox, G. J., and Hudson, L.: J. Nutrition **2**:271, 1930.

adequately explained (Griffith [3b]), but the assumption that renal changes resulting from supplements of small amounts of cystine are due to choline deficiency and that the requirement of choline in the diet of young rats depends in part on the amount of methionine and of cystine in the protein constituent of the food mixture is abundantly supported.

The extent of choline deficiency in rats on various diets was studied by Griffith, who noted the gross appearance and the weight of the kidneys, the deposition of lipids in the liver and the rate of growth of the experimental animals. The present study has been inspired by the gross observations of Griffith and is based on rats used in his work. It was carried out to determine the microscopic changes in the kidneys during the development of the renal lesions and particularly the processes of repair and partial recovery which occur after the initial acute congested phase.

MATERIALS AND METHODS

Rats 21 to 26 days of age, weighing 38 to 42 Gm., were placed in raised cages and fed experimental diets ad libitum. At the end of each experimental period, they were killed by decapitation, and the kidneys were removed and fixed in Zenker's solution with formaldehyde.[8] Sections were stained with hematoxylin and eosin, Giemsa's stain or Heidenhain's azocarmine–aniline blue as described by McGregor.[9] Both kidneys of each of 125 rats were studied. Forty per cent of these were obtained from animals which were on the choline-deficient diet from three to ten days, during which time the acute stage of renal degeneration develops. The remaining 60 per cent were obtained from animals which had survived the acute phase and were killed after periods of from ten to four hundred and eighty days. The latter group included:

1. Rats which had been maintained continuously on diet AC 32 or CG 3.

2. Rats which had been fed diet AC 39 for ten days and then Purina dog chow [10] for the remainder of the experiment.

3. Rats which were given diet AC 39 for seven days and then diet AC 32 or Purina dog chow until the close of the experiment.

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8. This is Zenker's solution prepared with neutral solution of formaldehyde U. S. P. instead of glacial acetic acid.

9. McGregor, L.: Am. J. Path. **5**:559, 1929.

10. The latest published analysis of the Purina dog chow is as follows: protein, 22.50 per cent; fat, 5.50 per cent; fiber, 3.75 per cent; ash, 7 per cent; carbohydrates (nitrogen-free extract), 50.25 per cent; moisture, 11 per cent; calcium, 1.75 per cent; phosphorus, 1 per cent; magnesium, 0.11 per cent; potassium, 0.70 per cent; soluble chlorides (as NaCl), 1.25 per cent; manganese, 75 parts per million; iron, 180 parts per million; copper, 8 parts per million; cobalt, 0.10 parts per million; vitamin D, 2.30 U. S. P. XI units per gram; vitamin A, 8.50 U. S. P. XI units per gram; carotene, 1.70 parts per million; thiamine chloride (B_1), 4 parts per million; riboflavin (B_2), 4.75 parts per million; pantothenic acid, 10 parts per million; niacin, 35 parts per million.

The experimental diets are outlined in the accompanying table. The control animals used in this study were given the basal experimental diets with 0.3 per cent choline chloride added.

In the rats which received diet AC 39 for ten days and then Purina dog chow, the renal degeneration was confirmed by laparotomy at the end of the first ten day period. These animals were killed between four and sixteen months after the beginning of the experiment.

GROSS OBSERVATIONS

The gross appearance of the kidneys affords a criterion of the severity of the effect of the experimental diet, although there is considerable variation in the responses in different rats. The onset of the signs of choline deficiency is extremely rapid in young rats, as is indicated by the occurrence of fatty infiltration of the liver within two to

Composition of the Diets

	Percentage in Given Diet		
	AC 39 *	AC 32	CG 3
Purified casein	18	15	15
Gelatin	0	0	3
Cystine	0	0.3	0.1
Cholesterol	0	0	0.5
Lard	20	9	20
Sucrose (commercial)	54.9	62.6	49.3
Dried brewers' yeast	0	6	6
Salt mixture †	4	4	4
Calcium carbonate	1	1	0
Agar	2	2	2
Natola ‡	0.1	0.1	0.1

* Water-soluble vitamins were supplied by daily oral administration of 0.02 mg. of thiamine hydrochloride, 0.02 mg. of riboflavin, 0.02 mg. of pyridoxine and 0.5 mg. of nicotinic acid.

† The salt mixture was described by P. B. Hawk and B. L. Oser (Science 74: 369, 1931).

‡ This is a halibut liver oil fortified with other fish liver oils rich in vitamin D.

three days. The renal lesions appear as early as the fourth day on the diet containing added cholesterol and on the sixth day on the low choline diets. At the beginning of the congestion the surface of the kidney appears blotched. The entire superficial zone gradually becomes engorged, and the kidney appears greatly enlarged and purplish red. In the cases in which the condition is most severe, gross sections of the kidney show a deep red peripheral zone throughout the entire cortex. In cases in which the kidneys are less severely affected, sections may show the peripheral zone of the cortex spotted by congested areas. Animals which do not die during the acute stage pass through a spontaneous "recovery," which is almost as spectacular as the onset of the pathologic state. In this phase the congestion disappears, although the fatty condition of the liver and signs of renal damage persist. Recovery is usually initiated by the tenth day, and the spontaneous repair is similar to that which follows administration of choline immediately after the first appearance of the renal damage. In the early phases of recovery,

the kidneys show alternating areas of congestion and discolored areas without congestion. As the congested areas disappear, the kidneys remain enlarged and appear yellowish and bloodless if the previous acute stage was moderate and chalky yellowish white if the acute stage was severe. Kidneys of the latter type are frequently covered with irregular masses of white material and are recorded as "frosted." Recovered kidneys examined months after the acute phase are usually of normal weight, sallow brown, irregular in shape and roughened by grooves or depressions which mark the positions of scar tissue. The white deposit of frosted kidneys persists for as long as two months.

MICROSCOPIC OBSERVATIONS DURING FIRST TEN DAYS: THE ACUTE REACTION

Vascular Changes.—The gross appearance of the affected kidneys is due chiefly to the congestion of the cortical capillaries (fig. 1) and the capsular blood vessels. Engorgement causes the capsule to become greatly thickened, and the tubules in the peripheral zone of the cortex appear to be surrounded by a network of engorged vessels since the connective tissue is almost obliterated. The peripheral glomeruli often appear normal, but in all severely congested kidneys, especially those from animals on diet AC 39, the glomeruli also are distended with blood. Hemorrhage is slight or absent in most of the kidneys. Fibrin has been demonstrated, during the acute stage, in the capsule and sometimes at the periphery of the cortex. This suggests that hemorrhage originates entirely from capsular blood vessels. No red blood corpuscles have been observed in the tubules. The walls of the congested cortical vessels and of the large renal vessels exhibit no degeneration except in the kidneys, in which the entire peripheral zone of the cortex is necrotic. Such kidneys exhibit massive destruction of the vascular system in the peripheral zone of the cortex.

Tubular Changes.—Affected kidneys vary considerably in the amount but not in the type of tubular degeneration. In the areas of vascular congestion, necrosis involves either small or large groups of convoluted tubules (fig. 1). Kidneys of animals on diet AC 39 uniformly show necrosis of tissues in a peripheral band situated just beneath the surface of the cortex. Irrespective of the amount of necrosis, the structural configuration of the cortex is retained during the acute stage. At the beginning of necrosis, pyknosis and nuclear fragmentation of the cells in the renal tubules are seen, especially in kidneys of animals on diet AC 39. In the kidneys of animals on diet AC 32 the process of degeneration is more commonly initiated by fading of the cells, particularly of the nuclei.

Many less affected tubules at the periphery are swollen, and the cytoplasm of the epithelial cells undergoes albuminous degeneration as far as the nucleus. The destruction of the cytoplasm results in the

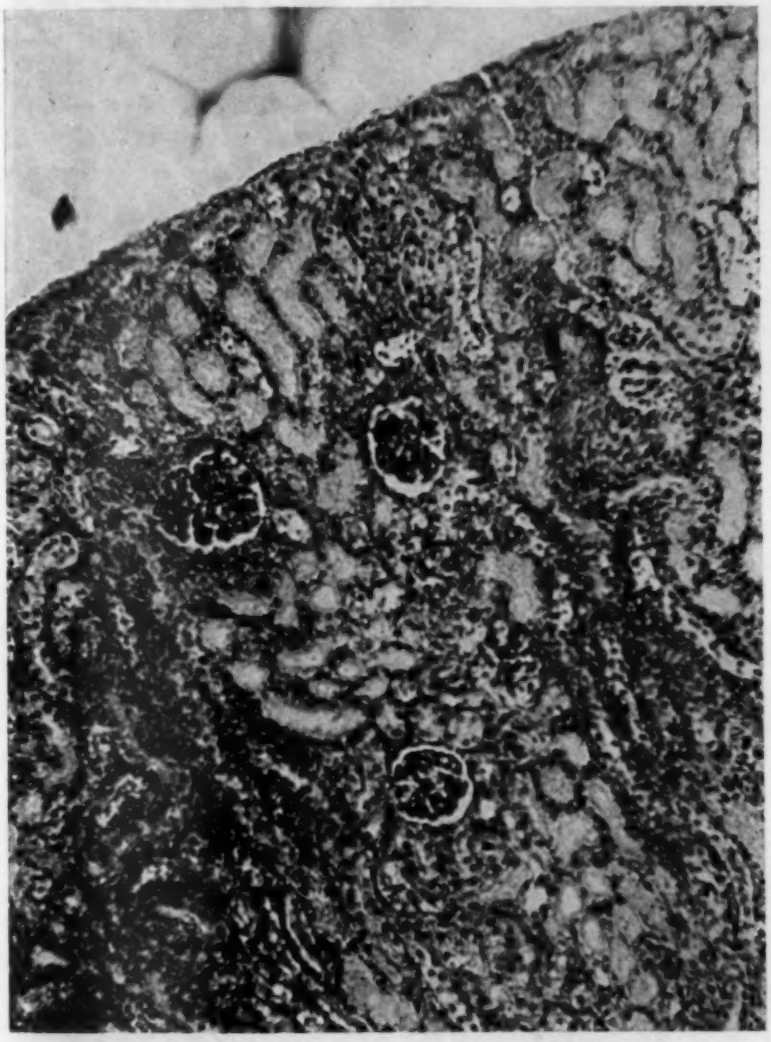

Fig. 1.—Peripheral zone of the cortex of the kidney during the acute stage of choline deficiency, showing congestion of the cortical capillaries and necrosis of the cortical tubules. The capsule of the kidney was removed before fixation. The rat was fed diet AC 39 for seven days. Hematoxylin and eosin; × 165.

formation of a fine granular precipitate in most tubules, but in some, large acid-staining hyaline globules (hyaline droplet degeneration) are

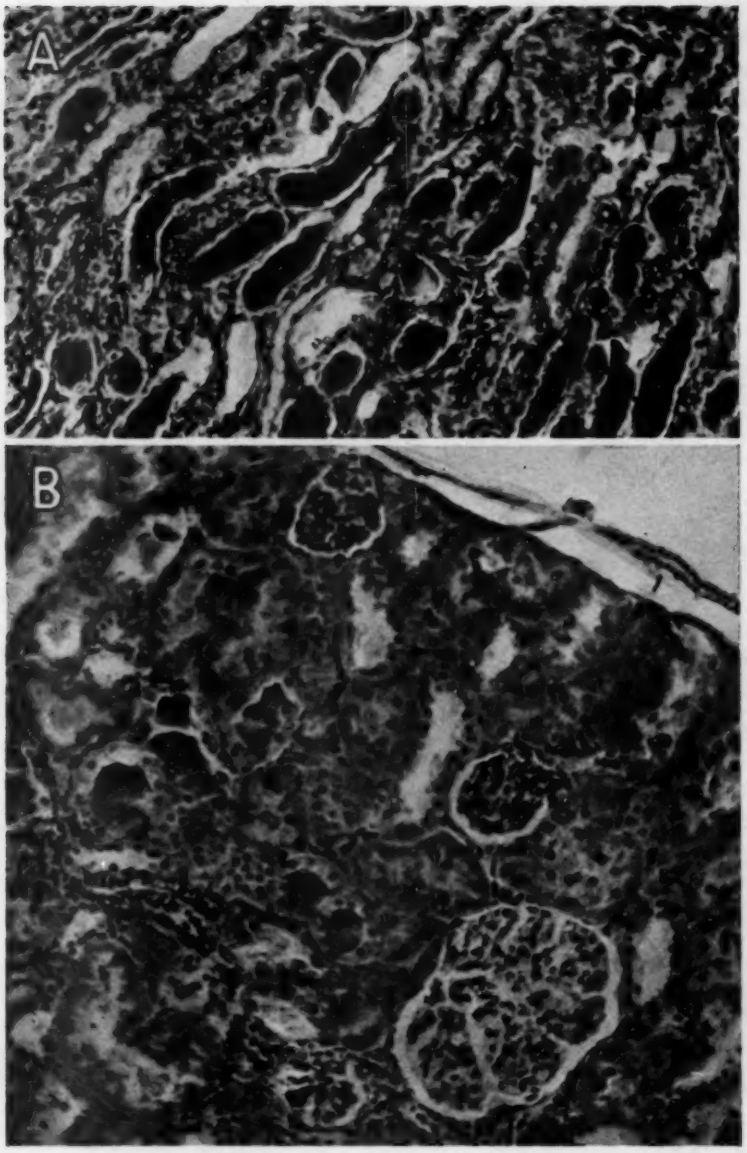

Fig. 2.—*A*, tubules in the deep part of the renal cortex filled with hyaline casts. The rat was fed diet AC 32 for ten days. Giemsa's stain; × 165. *B*, peripheral zone of the cortex of a recovered kidney with small renal corpuscles, casts and albuminous degeneration of tubules. The rat was maintained on diet AC 39 for ten days and then on Purina dog chow for the remainder of the experimental period of sixteen months. Hematoxylin and eosin; × 360.

formed, which fill the lumens. In the cortical labyrinths, groups of enlarged, empty tubules with basal remains of epithelium show the effects of early granular degeneration. Bowman's capsules are affected similarly in the most severe conditions, but the cytoplasmic degeneration is not so extensive and there is little debris in the lumens. Frequently the tubules with hyaline droplet degeneration are edematous, and both the nuclei and the degenerating cytoplasm are displaced centrad. Occasionally epithelial cells, singly or in small groups, are present in the cytoplasmic debris in the lumens, but desquamation is not an important process in tubular degeneration. In the peripheral half of the cortex only scattered tubules are filled with hyaline casts. Casts are always present in the tubules in the inner zone of the cortex (fig. 2*A*) and the outer zone of the medulla in *all* affected kidneys. The papillary ducts and tubules of the renal pyramids contain casts only in the most severely affected animals. Paradoxically, numerous uninjured cells in the cortical tubules are observed in mitosis in spite of the degeneration going on about them.

MICROSCOPIC OBSERVATIONS FROM THE TENTH DAY TO THE FOURTH MONTH, AFTER THE ACUTE DEGENERATIVE STAGE

Regeneration of the renal tissue begins between the seventh and the tenth day if the acute reaction has been reached early; otherwise it begins very soon thereafter. In animals in which the peripheral degeneration is patchy, the repair is limited largely to wedge-shaped areas; if extensive degeneration has occurred, the entire periphery is involved in the reparative process. Repair in other parts of the kidney does not show the transformation that occurs in the peripheral necrotic zones.

Disappearance of Congestion and Hemorrhage.—Blood moves out of the congested vessels both in the capsule and in the cortex soon after the beginning of recovery. Blood corpuscles which are freed in the connective tissue by hemorrhage are taken up by macrophages. Early in the process of recovery macrophages filled with red corpuscles are unusually abundant in the capsules of some kidneys, and throughout the period of repair some may be found in the scar tissue at the periphery of the cortex. In some animals continuously on diet AC 32 a second period of cortical congestion develops about the eighteenth to the twentieth day, which lasts for only a short time. The kidneys again have a deep red appearance, although stationary body weights also indicate the second response to the diet. With this exception, congestion is never again as great as during the acute response.

Fibrosis.—Immediately after the acute stage, the proliferation of interstitial tissue results in the formation of a richly cellular tissue,

which invades the areas of degeneration and encloses the remains of degenerated tissue as well as parts beginning to regenerate. Here and there some infiltration of the tissue by monocytes and occasionally by neutrophils may be observed. The only fibrosis in the deeper parts of the cortex follows the medullary rays, where the dense cellular tissue obscures the tubular structure unless the tubules are distended by casts.

Calcification.—Degenerating tubules become calcified about the tenth day in some kidneys of animals on diets AC 39 or CG 3, but calcification has not been observed until after the tenth day in animals on diet AC 32. The first calcified tubules are found at the periphery of the cortex or internal to the zone of cortical necrosis; later all calcified remains of tubules are located in the scar tissue.

Other Tubular Changes.—Necrotic tubules or large areas of complete necrosis are removed by lysis. After the beginning of lysis the structural configuration in the degenerated cortical tissue is lost.

The recovery of tubules with granular degeneration helps most to reestablish the peripheral zone of the cortex. These tubules merely lose their swollen condition, and the cytoplasmic degeneration becomes less extensive. The appearance of this part of the cortex after the recovery of these tubules is almost identical with that of the normal kidney.

Casts usually leave the tubules in the early stages of regeneration. After they disappear, the epithelium of the tubules is flattened, and the prominent lumens sometimes are occupied by a loose reticulum. In the pyramids in most of the kidneys, enlargement produced by casts causes no particular damage except the great thinning of the walls, which sometimes are broken as a result of distention. Under these conditions the ruptured tubules may appear to fuse.

Changes in Renal Corpuscles.—Fibrosis external to the parietal layer of Bowman's capsule occurs around peripherally situated renal corpuscles. In sections, the corpuscles exhibit "rings" of connective tissue about them. Besides pericapsular fibrosis, renal corpuscles in the outer zone of the cortex which have shown no appreciable changes during the acute response are often found to be reduced in size during the early repair stage. Presumably, they do not grow as much as the corpuscles in the deeper portion of the cortex and remain small. The small corpuscles are clustered in the developing scars, some of them almost against the capsule in a position taken after the disappearance of the degenerated tubules. Thicker epithelium in Bowman's capsule also is not uncommon in the renal corpuscles which have passed through the acute reaction without apparent damage.

Uninjured cells in partially necrotic tubules offer the means for partial restoration of degenerated tubules in the scar tissue. As tubules are rebuilt the necrotic parts are pushed aside. The regenerating tubules are small in diameter, with or without lumens and widely separated in the scar tissue. Their cells exhibit prominent spherical nuclei and basophilic cytoplasm.

Kidneys from animals which continued to receive the deficient diets after the ten day period show evidence of less rapid recovery as compared with those of animals which were given diets with adequate amounts of choline immediately after the acute reaction. In the former, although the peripheral part of the cortex shows fibrosis and breakdown of necrotic tissue, the convoluted tubules deeper in the cortex may remain enormously swollen and exhibit severe granular degeneration. Casts likewise disappear slowly or not at all, especially in the tubules of the inner zone of the cortex and the outer zone of the medulla.

MICROSCOPIC OBSERVATIONS AT FOUR TO SIXTEEN MONTHS: LATE RENAL CHANGES

In animals which have had choline restored to the diet, recovery is completed in four months except for the peripheral wedge-shaped areas, which are much reduced in size and flattened against the capsule. These areas include necrotic and calcified tubular remnants, small renal corpuscles, regenerating tubules, and scattered macrophages filled with red blood cells. At six months, the "wedges" disappear completely as the regenerated tubules and the small renal corpuscles are distributed in the adjoining tissue.

The recovered kidneys appear structurally similar to normal ones but are not entirely free of chronic degeneration. The degree to which regeneration is possible is determined by (1) the initial severity of the acute reaction, (2) the persisting effects of the primary lack of choline and (3) the effect of continued lack of choline after the acute response. The oldest recovered kidneys (sixteen months) vary with regard to the types and the extent of chronic degeneration which they exhibit.

Small renal corpuscles (fig. 2*B*) in the outer zone of the cortex are found as commonly as any of the other renal changes. Granular degeneration of convoluted tubules is also common. The tubules may be only slightly enlarged, but the inner borders of the cells continue to fragment and cellular debris is found in the lumens. Retention of casts in some instances causes the tubules to become distended until they are cystlike (fig. 3*A*). The diameter is much greater than that of any tubules filled with casts during the early acute degeneration. Albuminous casts also collect in Bowman's capsules and cause them to become

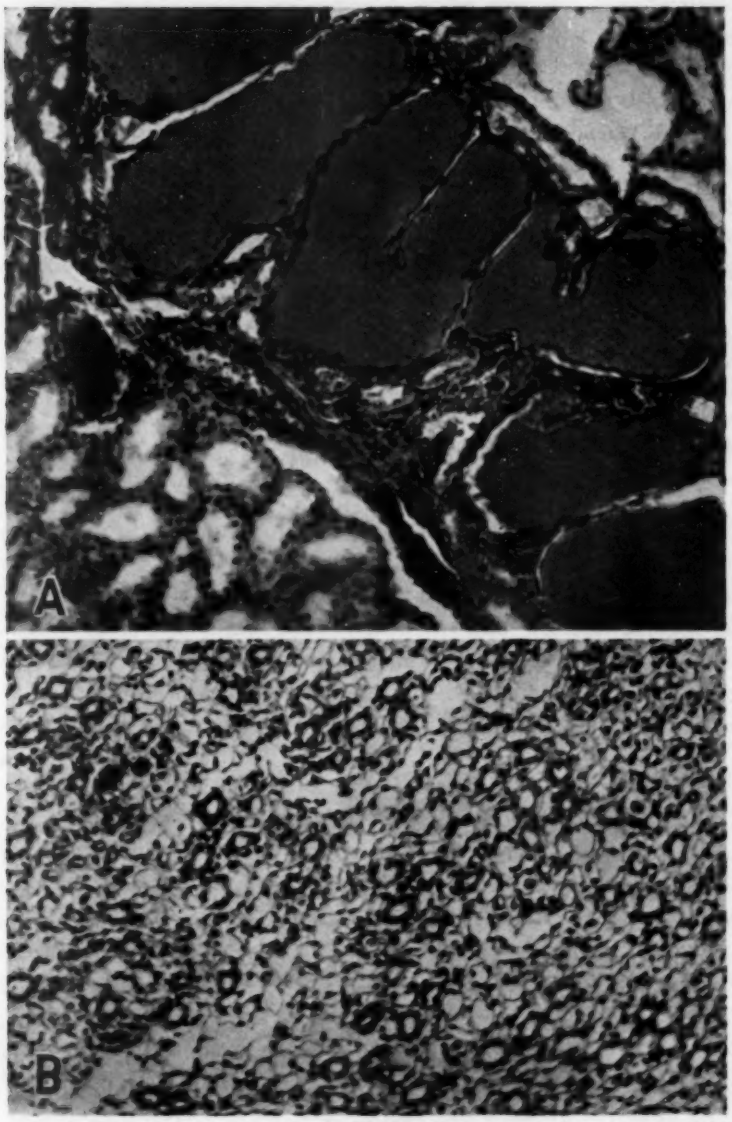

Fig. 3.—*A*, tubules of the cortex greatly distended with casts. Other tubules are enlarged and show chronic albuminous degeneration. The rat received diet AC 39 for ten days and then Purina dog chow for the remainder of the experimental period of sixteen months. Hematoxylin and eosin; × 165. *B*, enlarged and thin-walled tubules in a renal pyramid after the disappearance of casts in a spontaneously recovered kidney. The rat was fed diet AC 32 for six months. Hematoxylin and eosin; × 165.

balloon-like in appearance. In such corpuscles the glomeruli usually are either normal in diameter or slightly contracted. In some convoluted tubules the cells have an altered cytoplasmic-nuclear relationship. The cytoplasm in the outer parts of the cells is dense, acid staining and hyaline; the inner cytoplasm, which contains the nuclei, is loose and reticulated. No particular significance can be assigned to this differentiation. Many of the tubules in which casts were present during the acute stage are still distended and thin walled months after the casts have disappeared (fig. 3 *B*).

The vascular system shows only the slightest evidence of chronic change. Local congestion of blood vessels in the peripheral zone of the cortex about the convoluted tubules or in the medulla in the vicinity of the distended tubules has been observed in several kidneys. A dearth of blood corpuscles in large but normal-appearing glomeruli of the peripheral zone of the cortex has also been observed. Partial avascularity, probably due to the closure of some vascular loops by endothelial proliferation, is seen occasionally in glomeruli.

COMMENT

In the chronic renal degeneration described in the foregoing section, the damage which results from necrosis of peripheral renal tubules has not been considered. Parts of renal tubules which have been isolated as a result of necrosis apparently do not degenerate. Their identification in sections is not readily made. Cystlike tubules might be regarded as isolated portions of incompletely degenerated tubules incapable of being freed of casts. Local distention of intact renal tubules by casts which makes them appear cystlike has also been described (Oliver [11]). The percentage of renal tubules which remain intact after massive necrosis cannot easily be determined, but it may be assumed that large numbers of tubules are partially destroyed.

The temporary stasis of the blood in the capillary vessels of the peripheral regions of the cortex and in the congested glomeruli permits little blood to leave the peripheral zone. New shunts provided by small cortical vessels in the vicinity are necessary to carry the blood from the region of degeneration to deeper efferent channels. After the acute reaction, the vascularity of the peripheral zone of the cortex in the absence of necrosis is reestablished without serious defects. Slight changes of the cortical vascular system that might be associated with the small glomeruli have not been noted. Permanent alterations develop when there has been extensive peripheral necrosis; then the new vascular

11. Oliver, J.: Architecture of the Kidney in Chronic Bright's Disease, New York, Paul B. Hoeber, Inc., 1939.

channels that grow into the zones of fibrosis probably subserve only a nutritive function. It is impossible for them to act as part of an excretory mechanism because tubular regeneration in the necrotic zone is incomplete and the vascular relations are imperfect.

To what extent tubular degeneration and vascular congestion are interdependent cannot be stated definitely. At the beginning of the present study the tubular degeneration seemed to be associated with the vascular changes. Certain observations indicate that early tubular degeneration and vascular disturbance are somewhat separate entities. The appearance of tubular necrosis before the vascular congestion is at its peak seems to support this assumption. The relationship of renal degeneration to other organic changes, particularly the fatty infiltration of the liver, has not been determined.

Renal degeneration of a type similar to that described for choline deficiency has been observed before in animals given experimental diets. Newburgh and co-workers and several other investigators found that diets high in protein produce definite renal injury. The kidneys of rabbits given diets consisting solely or in part of egg white, casein or soybeans (Newburgh [12]) were always much enlarged and showed marked engorgement, edema and severe tubular injury. A diet of 27 per cent or more of meat (Newburgh and Clarkson [13]) resulted in a somewhat similar picture of renal disease so far as the tubules were concerned, but the atrophy of the tubules was not accompanied by significant changes in the glomeruli or blood vessels.

Since the diets used in these early investigations obviously were inadequate, they were subjected to much criticism (Mitchell [14]; Bischoff [15]). Among those who repeated the experiment, Polvogt, McCollum and Simmonds,[16] after giving adequate diets high in protein (31 to 41.3 per cent) to old rats, were able to confirm Newburgh's findings. They observed no hemorrhage into Bowman's capsules or into the tubules. Later Newburgh and Curtis [17] also carried out studies in rats in which they used carefully prepared diets with a high protein content with results that were essentially similar to those which they had previously reported. Studies of Blatherwick and Medlar [18] and Medlar and Blatherwick [19] in general corroborate the finding of the

12. Newburgh, L. H.: Arch. Int. Med. **24**:359, 1919.
13. Newburgh, L. H., and Clarkson, S.: Arch. Int. Med. **32**:850, 1923.
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15. Bischoff, F.: J. Nutrition **5**:431, 1932.
16. Polvogt, L. M.; McCollum, E. V., and Simmonds, N.: Bull. Johns Hopkins Hosp. **34**:168, 1923.
17. Newburgh, L. H., and Curtis, A. C.: Arch. Int. Med. **42**:801, 1928.
18. Blatherwick, N. R., and Medlar, E. M.: Arch. Int. Med. **59**:572, 1937.
19. Medlar, E. M., and Blatherwick, N. R.: Am. J. Path. **13**:881, 1937.

previous investigators mentioned, that diets of high protein content produce renal degeneration in rats.

Newburgh explained the renal damage produced by high protein diets as due to the toxic effects of increased end products of protein metabolism. On the basis of this assumption, Newburgh and Marsh [20] were among the first to study the effects of administering amino acids to animals and demonstrated that arginine, aspartic acid, lysin, histidine, tyrosine, tryptophan and cystine are nephrotoxic. The toxic action of cystine especially was described by Curtis and Newburgh [21] and Cox, Smythe and Fishback.[22]

The importance of a vitamin B deficiency of the diet (rather than the protein or the amino acid fed) has become apparent in studies on the prevention of renal damage by feeding yeast. Hartwell [6] reported that the vessels of kidneys of rats fed a diet high in the protein edestin were engorged and that this condition was prevented by feeding the rats a yeast product, marmite, with the edestin. Cox and Hudson [7] also found that an unknown factor of the vitamin B complex in yeast counteracts the toxic action of cystine on the kidneys. After Griffith and Wade [2] demonstrated that choline prevents the renal degeneration observed on diets similar to those used in the aforementioned experiments, choline or some other source of labile methyl (Griffith and Mulford [23]) appeared to be the factor in question in the B_2 complex. György and Goldblatt [24] confirmed this finding and suggested that pyridoxine (B_6) plays a role in choline metabolism.

Renal lesions caused by vasopressor drugs presumably producing localized vasospasm (Byrom [25]; Penner and Bernheim [26]) have been reported, which appear to be similar to those produced by the feeding of experimental diets.

The renal alterations observed in animals fed high protein or high cystine diets by other investigators have been compared mainly with those observed in human nephritis or nephrosis. But the pathologic conditions of the human kidney which more nearly seem to resemble those of choline deficiency are symmetric cortical necrosis (Duff and

20. Newburgh, L. H., and Marsh, P. L.: Arch. Int. Med. **36**:682, 1925.
21. Curtis, A. C., and Newburgh, L. H.: Arch. Int. Med. **39**:817, 1927.
22. Cox, G. J.; Smythe, C. V., and Fishback, C. F.: J. Biol. Chem. **82**:95, 1929.
23. Griffith, W. H., and Mulford, D. J.: J. Am. Chem. Soc. **63**:929, 1941.
24. György, P., and Goldblatt, H.: J. Exper. Med. **72**:1, 1940.
25. Byrom, F. B.: J. Path. & Bact. **45**:1, 1937.
26. Penner, A., and Bernheim, A. I.: Arch. Path. **30**:465, 1940.

More[27]), acute ischemic renal necrosis (Penner and Bernheim[26]), preeclamptic toxemia (Byrom[25]) or the tubular disease described by Bell.[28] From the point of view of etiology these diseases are not clearly understood. The causative agents are regarded as toxins, vascular spasm or vascular lesions. The tubular degenerations in these diseases and in the choline-deficient animals are strikingly similar. Although such human renal lesions undoubtedly are similar histologically to those resulting from dietary deficiency in experimental animals, they should not at the present time be regarded as entirely comparable to those produced experimentally in animals. It may be possible, however, by further study to interrelate the results obtained from the study of choline deficiency in animals with the causation of certain types of human renal disease.

SUMMARY

In young rats fed choline-deficient diets an acute type of renal degeneration develops which may result in mild renal disease. The primary vascular change during the acute stage is congestion of the peripheral cortical capillaries and capsular blood vessels and sometimes of the glomerular blood vessels. Hemorrhage, if present, occurs only in the capsule and at the periphery of the cortex. Peripheral cortical tubules undergo necrosis or granular or hyaline droplet degeneration. Hyaline casts are always abundant in tubules of the inner zone of the cortex and the outer zone of the medulla. Calcification of degenerated peripheral tubules occurs in the most severely affected kidneys.

Recovery after the acute stage is remarkably complete.

In the oldest recovered kidneys the chronic results of choline deficiency appear as a reduction in the size of glomeruli, slight local congestion, chronic granular degeneration, retention of casts and abnormal dilatation of thin-walled tubules.

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27. Duff, G. L., and More, R. H.: Am. J. M. Sc. **201**:428, 1941.

28. Bell, E. T.: A Textbook of Pathology, ed. 3, Philadelphia, Lea & Febiger, 1938, pp. 14 and 416.

VARIATION OF VITAMIN A FLUORESCENCE IN THE CYCLIC CHANGES OF THE OVARY

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AND
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Classic histologic descriptions of the ovary have improved knowledge of estrogen and progesterone.[1] Many points, however, are still obscure; the utilization of a new histologic method may be of value in further clarifying the problems.

In the past few years the demonstration of vitamin A in tissues by means of fluorescence microscopy has been described.[2] This technic offers a new way of differentiating lipoids in tissues.

The purpose of this paper is to report a study of the distribution of vitamin A fluorescence in the ovaries of children, of women in different stages of the menstrual cycle and of women in pregnancy or at the climacterium, which was undertaken in order, first, to examine the relationship between vitamin A and estrogen and progesterone and, second, to investigate the structure of the ovary by a new method.

In a previous paper a description of the distribution of vitamin A fluorescence in the ovary based on postmortem material was given by Popper.[3] The material of the present study except for the children's ovaries was obtained from fresh surgical material, in cases in which a better menstrual history was available.

Vitamin A has a green fluorescence in ultraviolet rays, which is destroyed by radiation. The same green fading fluorescence is imparted by some lipoids in frozen sections of human and animal tissue. That it

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1. Meyer, R., and Ruge, C.: Zentralbl. f. Gynäk. **37**:50, 1913. Novak, E.: Gynecological and Obstetrical Pathology with Clinical and Endocrine Relations, Philadelphia, W. B. Saunders Company, 1940. Pratt, J. P.: Arch. Path. **19**:380, 1935.

2. von Querner, F.: Klin. Wchnschr. **14**:1213, 1935. Popper, H.: Proc. Soc. Exper. Biol. & Med. **43**:133, 1940.

3. Popper, H.: Arch. Path. **31**:766, 1941.

is due to vitamin A has been proved by histochemical reactions and experiments on avitaminotic and hypervitaminotic rats.[4]

A relationship of vitamin A to reproduction was assumed.[5] Vitamin A deficiency causes continuous cornification of the vaginal epithelium independent of the normal estrus cycle, and this phenomenon was utilized as a biologic assay for vitamin A.[6] It is, however, a local change and not due to altered ovarian function. That excessive doses of vitamin A suppress the menstrual cycle [7] has not been confirmed.[8] The question whether vitamin A or carotene is essential for the production of estrus suggested experiments on castrated female mice on a vitamin A-deficient diet, in which estrus was produced if estrogens and carotene were injected but not if only one of these substances was injected.[9]

MATERIAL AND METHODS

The accompanying table indicates the material studied. Cases without ovarian structural changes were selected. In the group of mature nonpregnant and that of climacteric women both ovaries and the endometrium were studied in each case and the findings correlated with the menstrual history. The age in the first group was between 30 and 43 years. In most cases of pregnancy the age was lower.

The tissues were fixed not longer than eight hours in a solution of formaldehyde U. S. P. diluted 1 : 10. Frozen sections were examined under the fluorescence microscope for vitamin A, in unstained sections, and for fat, in sections stained by phosphin 3R. Details of the methods used are given in a previous paper.[3] The fat solubility of the fluorescent details was ascertained by treating the sections with alcohol or acetone for three minutes. Vitamin A fluorescence disappears after this treatment.

In addition routine stains (sudan 3 and hemalum-eosin) were made.

The fluorescence photomicrographs were taken with a 35 mm. camera and adapter, an ultraspeed film and forty seconds' exposure being used.[10]

RESULTS

Children's Ovaries.—In ovaries of newborn full term and premature infants, the presence of little if any fat and absence of vitamin A were

4. Popper, H., and Greenberg, R.: Arch. Path. **32**:11, 1941.

5. (*a*) Browman, L. G.: Am. J. Physiol. **125**:335, 1939. (*b*) Evans, H. M.: J. Biol. Chem. **77**:651, 1928. (*c*) Mason, K. E., and Ellison, E. T.: J. Nutrition **9**:735, 1935; **10**:1, 1935.

6. (*a*) Baumann, C. A., and Steenbock, H.: Science **76**:417, 1932. (*b*) Evans, H. M., and Bishop, K. S.: Anat. Rec. **23**:17, 1922. (*c*) Hohlweg, W., and Dohrn, M.: Biochem. J. **30**:932, 1936.

7. Sherwood, T. C.; Brend, M. A., and Roper, E. A.: J. Nutrition **11**:593, 1936.

8. Burill, M. W., and Greene, R. R.: Endocrinology **28**:765, 1941.

9. von Euler, H.; Zondek, B., and Klussmann, E., cited by Clausen, S. W.: J. A. M. A. **111**:144, 1938.

10. Popper, H., and Elsasser, M.: Canad. M. Technol. **3**:45, 1941.

demonstrable in the stroma, the primordial follicles or the ova. The collagenous fibers gave a striking blue and the primordial follicles a dim blue fluorescence.

At five months of postnatal life fine lipoid droplets appeared in the cortex, with distinct accumulation around the primordial follicles (fig. 1). These droplets emitted a bright, quickly fading vitamin A fluorescence. After fading of the green fluorescence, some revealed a red-brown ultraviolet-stabile fluorescence. In some ovaries this nonalcohol-soluble and partly acetone-soluble pigment was immediately visible without any vitamin A fluorescence. In addition, lipoids were seen in granulosa and theca cells of the primordial follicles and the brown-red fluorescent pigment, less frequently, in the latter.

As the primordial follicle developed into the early graafian follicle, fat and vitamin A fluorescence increased. The theca cells revealed a rather slowly fading vitamin A fluorescence, imparted by medium-sized lipoid droplets, some of them with slight double refraction. The vitamin A fluorescence of the granulosa cells was brighter, faded quickly and was imparted by fine lipoid droplets. It was accumulated particularly in the cumulus oophorus (fig. 1 *B*); the ovum itself was free of vitamin A fluorescence despite occasional traces of fat. Red-brown pigment, if present, was seen in small amounts in the theca cells.

With atresia of the follicle, fat and vitamin A fluorescence of the theca cells increased, being imparted by large droplets, which often filled the entire cell and sometimes showed double refraction (fig. 1 *C*). This vitamin A fluorescence was not bright and faded rather slowly. After the fading, some red-brown pigment was often visible. In the fibrotic center of the corpora atretica nondouble-refractile fine fat droplets were seen without vitamin A fluorescence. The stroma around the corpora atretica was often rich in fine fat droplets with or without vitamin A fluorescence. The starlike shape of larger corpora atretica was sharply outlined by the vitamin A fluorescence (fig. 1 *D*). With further involution of the corpora atretica, the vitamin A fluorescence gradually disappeared. Finally the fat disappeared also and the red-brown pigment increased. In the final stages of involution the starlike corpora atretica were outlined by cells with red-brown pigment.

Ovaries in the Reproductive Stage.—Characteristic changes occur during the menstrual cycle. In the ovaries examined (from women in the third decade of life) only few primordial follicles were seen. The maturing follicles showed a picture similar to that in the children's ovaries. The picture does not change until after the rupture of the graafian follicle. In a recently ruptured follicle, where the corpus luteum was just forming, the same distribution of vitamin A fluorescence was still noted.

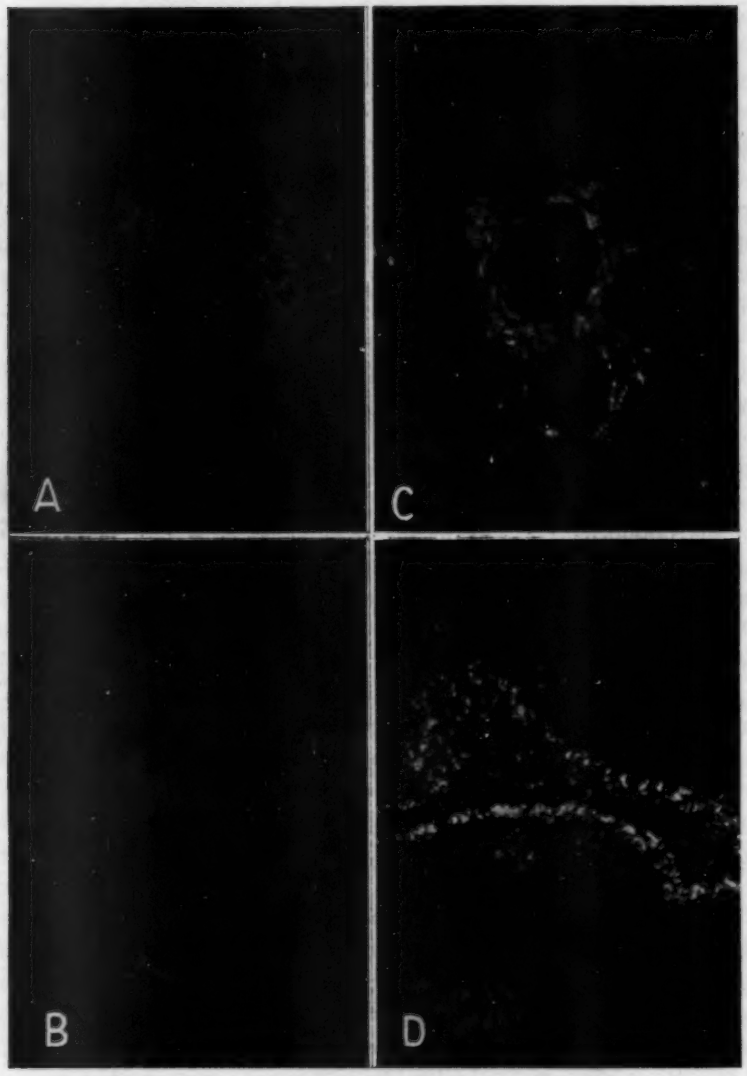

Fig. 1.—Fluorescence photomicrographs: *A*, cortex of the ovary of a child of 1 year, showing fine lipoid droplets with vitamin A fluorescence in the stroma. *B*, maturing follicle in a child's ovary, showing fine lipoid droplets with bright, quickly fading vitamin A fluorescence in the granulosa cells and dimmer, more slowly fading fluorescence in the theca cells. *C*, follicle in beginning atresia in a child's ovary, showing vitamin A fluorescence of the theca cells. *D*, corpus atreticum in a child's ovary stained with phosphin 3R, showing a strong fat reaction of the proliferating theca cells. Fine lipoid droplets without vitamin A fluorescence are present in the center. The droplets in the surrounding stroma reveal vitamin A fluorescence.

(*a*) Proliferating Corpus Luteum. In the very early corpus luteum fine droplets in the granulosa cells imparted a quickly fading vitamin A fluorescence (fig. 2 *A*). In addition, the cytoplasm showed some green, very slowly fading fluorescence, which disappeared also on treatment with acetone. The theca cells showed little fat and vitamin A fluorescence. With further development, the fine fat droplets in the proliferating granulosa cell layer increased in number and slightly in size, showing a brighter, quickly fading vitamin A fluorescence. They were irregularly distributed in the cytoplasm, which itself revealed more of the dull green fluorescence. The fibrinous center showed a moderate blue fluorescence.

In the stage of vascularization the droplets increased even more in size and fluorescence and showed a tendency to line up along the edge of the cells. They were accumulated in the outer and central zone of the granulosa cell layer (fig. 2 *B*). The dim, slowly fading green fluorescence of the cytoplasm was very strong. After the fading, a dirty green-brown dim fluorescence persisted which was not removed by acetone. The center of the corpus luteum contained clotted blood and thus did not show any fluorescence except for scattered granulosa cell cords with many strongly fluorescent droplets.

(*b*) Mature Corpus Luteum. The vitamin A fluorescence was even more marked (fig. 2 *C*). The larger fat droplets were clearly distributed on the edge of the epithelioid cells, whose cytoplasm imparted a strong green, very slowly fading fluorescence. When the granulosa cells were adjacent to a capillary, the lipoid droplets were less regularly lined up or sometimes were even absent. The accumulation at the periphery and toward the center was marked. With fat stains, most but not all of the fluorescent droplets were demonstrated. Double refraction was absent. Various granules of irregular shape with green-brown ultraviolet-stabile fluorescence were seen in the cytoplasm of the granulosa cells.

The paralutein cells in the folds of the scalloped corpus luteum varied greatly in development, distribution and vitamin A fluorescence. Usually they manifested the fluorescence in a later period of development. Its intensity and the size of the fluorescent droplets increased gradually. The droplets always gave a fat reaction and usually slight double refraction. The paralutein cells often contained the red-brown, partly acetone-soluble pigment.

(*c*) Involuting Corpus Luteum. With beginning involution and shrinkage of the granulosa cell layer, the fluorescent droplets increased markedly in size, vitamin A fluorescence and fat content, the fat droplets becoming anisotropic (fig. 2 *D*). The droplets often filled the entire cell, and the green cytoplasmic fluorescence was hardly visible. After the

fading of the vitamin A fluorescence, brown fluorescence was visible in high intensity. With further involution, the vitamin A fluorescence disappeared and the brown fluorescence, increasing in strength, was immedi-

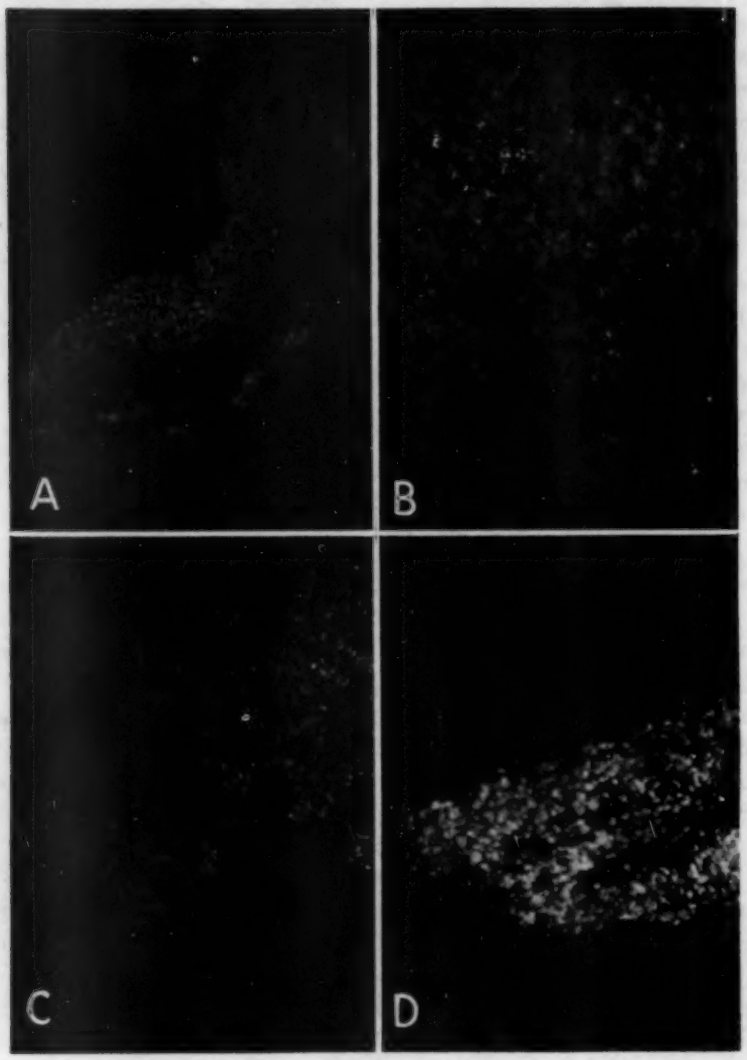

Fig. 2.—Fluorescence photomicrographs: *A*, very early corpus luteum. Note the fine droplets with vitamin A fluorescence in the granulosa cell layer. Some vitamin A fluorescence is seen in the theca cell layer. *B*, corpus luteum in the stage of vascularization (high power). Droplets with vitamin A fluorescence are present within the granulosa cells and are lined up on their edge. *C*, mature corpus luteum. The lipoid droplets are increased in number, size and fluorescence. *D*, corpus luteum in beginning involution. The lipoid droplets are larger and show strong vitamin A fluorescence.

ately apparent. It finally disappeared with formation of the corpus albicans.

The paralutein cells still showed great variations in their appearance and distribution; mostly they imparted strong double refraction. In the late stages of involution and even in the folds of the corpora albicantia theca cells were still recognizable by the ultraviolet-stabile red-brown pigment.

In the fibrous center many lipoid droplets were seen which were free of vitamin A fluorescence. Whereas the stroma about the maturing corpus luteum showed no characteristic fluorescence (except of small brown pigment granules), that about the involuting corpus luteum contained many fine lipoid droplets with vitamin A fluorescence (fig. 3 *A*). After the fading of this fluorescence, a red-brown fluorescence remained. The droplets decreased in number with increasing distance from the corpus luteum. The vitamin A fluorescence of the droplets was not related to the menstrual cycle but seemed to run parallel with that of the involuting corpus luteum itself.

The vitamin A fluorescence of the involuting corpus luteum did not depend on that of the recent corpus luteum, although it was most marked at the time when the vitamin A fluorescence started to increase in the new corpus luteum.

(*d*) Corpus Luteum in Pregnancy. The cytoplasm in the proliferated granulosa cell layer showed a diffuse, rather strong dull green fluorescence which faded very slowly and was removed by acetone. After this fading, a green-brown fluorescence persisted. In the later stages of pregnancy the dull green fluorescence was stronger. In early pregnancy (up to three months), irregularly scattered over the granulosa cell layer there were anisotropic large fat droplets, which imparted a bright vitamin A fluorescence and appeared as vacuoles in visible light. The bulk of the granulosa lutein cells was free of fat and vitamin A fluorescence (fig. 3 *B*). In later pregnancy the scattered fat-containing cells decreased in number. In the puerperium much anisotropic fat appeared with rather strong vitamin A fluorescence.

The paralutein cells varied markedly in vitamin A fluorescence. In some cases it was completely absent, whereas in others numerous doubly refractile lipoids with rather strong vitamin A fluorescence were present, strongly contrasting with the granulosa cells free of the fluorescence (fig. 3 *B*). The vitamin A fluorescence of the paralutein cells was not related to the duration of pregnancy. In many cases red-brown fluorescent pigment was visible.

(*e*) Fat Reaction of the Corpus Luteum. The fat reaction (sudan 3 or phosphin 3R) of the corpus luteum usually runs parallel with the vitamin A fluorescence. In pregnancy the fat reaction was restricted

to the strongly fluorescent large scattered droplets. Occasionally, however, vitamin A fluorescence was imparted by lipoid droplets in the granulosa cell layer which were not demonstrated by the ordinary fat

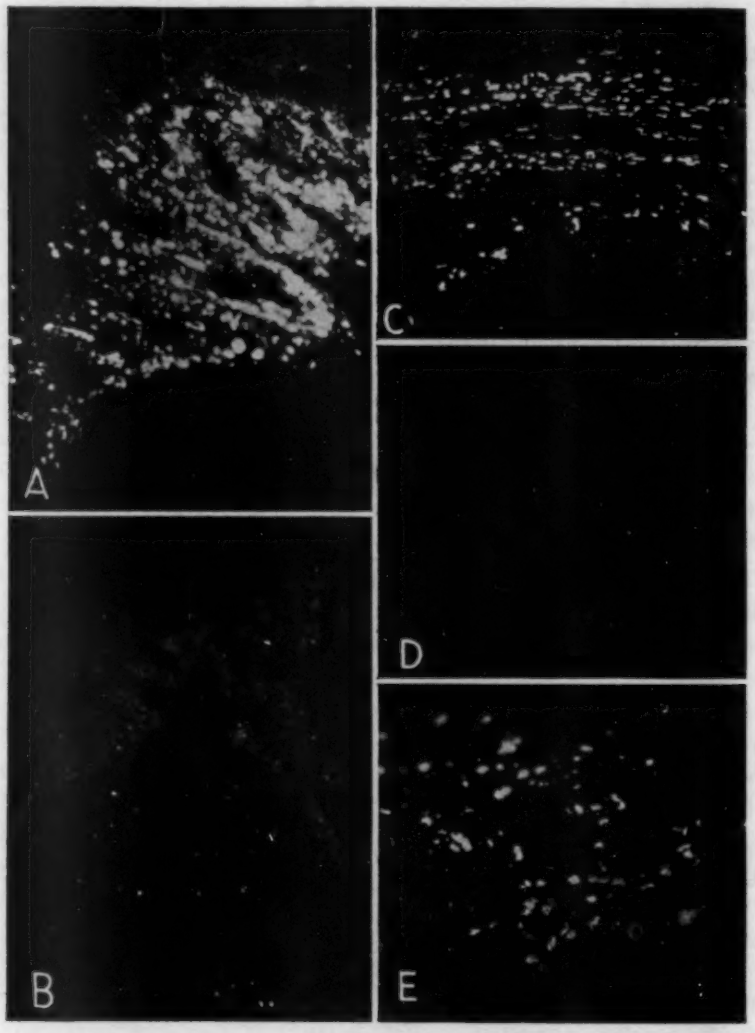

Fig. 3.—Fluorescence photomicrographs: *A*, involuting corpus luteum, showing strong vitamin A fluorescence of the large lipoid droplets in the corpus luteum and moderate fluorescence of the fine lipoid droplets in the surrounding stroma. The center is free of vitamin A fluorescence despite the presence of fat droplets. *B*, corpus luteum in pregnancy. The granulosa cell layer is free of vitamin A fluorescence except for some scattered large lipoid droplets. The paralutein cells are rich in vitamin A fluorescence. *C*, corpus atreticum. The proliferated theca cells are rich in vitamin A fluorescence. *D*, corpus atreticum. The theca cells show no vitamin A fluorescence but immediately a deep red-brown fluorescence. *E*, scattered theca cells with strong vitamin A fluorescence.

stains (10 cases). In 3 cases fat droplets were found in the granulosa without vitamin A fluorescence. In the theca cells the parallelism between fat reaction and vitamin A fluorescence was definite.

(*f*) Corpus Atreticum. The fibrous center revealed many fine fat droplets without any vitamin A fluorescence. The proliferating theca cells arranged in a starlike structure often showed a strong, moderately bright and relatively slowly fading vitamin A fluorescence imparted by large anisotropic fat droplets which usually filled the entire cell (fig. 3 *C*). After fading of this fluorescence, a light red-brown, partly acetone-soluble fluorescence persisted. In the involution of the corpora atretica the vitamin A fluorescence diminished, whereas after this fading a stronger red-brown fluorescence became visible. Finally the vitamin A fluorescence was completely gone and a strong red-brown fluorescence was immediately visible (fig. 3 *D*).

Fat reaction and double refraction often outlived the vitamin A fluorescence. The fluorescence of the fat droplets varied in the same corpus atreticum and even in the same cell, one drop being rich in vitamin A fluorescence and another revealing immediately the deep red-brown fluorescence.

The variation of the vitamin A fluorescence of the corpora atretica depends on three factors:

1. The size of the corpus atreticum. The earlier the atresia starts the smaller is the corpus atreticum and the lower the vitamin A fluorescence. Often small corpora atretica with immediate brown fluorescence are seen.
2. The age of the corpus atreticum. The older it is (as characterized by a more starlike shape) the lower the vitamin A fluorescence.
3. The degree of vitamin A fluorescence in the corpus luteum and thus the menstrual cycle. The more vitamin A fluorescence that is seen in the mature (not the involuting) corpus luteum, the more is imparted by the corpus atreticum (fig. 4). In the absence of corpora lutea (irregular menstruation) or of their vitamin A fluorescence (pregnancy) the corpora atretica show immediately the deep brown fluorescence.

The stroma about the corpora atretica, in contrast to that of the involuting corpus luteum, showed vitamin A fluorescence in only a few cases.

In the final dissolution of the corpora atretica the theca cells were scattered but still stood out rather distinctly by their strong red-brown ultraviolet-stabile fluorescence, which was partly soluble in acetone. Occasionally they contained doubly refractile lipoid droplets with or without vitamin A fluorescence (fig. 3 *E*). The vitamin A fluorescence

of the scattered theca cells was apparently inversely proportional to the vitamin A fluorescence of the involuting corpus luteum. The stroma around the scattered theca cells contained red-brown fluorescent granules without vitamin A fluorescence.

(*g*) Follicular Cysts. In some of the ovaries examined, follicular cysts were seen in various stages of development. In the granulosa cell layer fine isotropic fat droplets with quickly fading bright green vitamin A fluorescence were seen. The theca cells revealed larger, somewhat doubly refractile droplets with less bright, more slowly fading vitamin A fluorescence. Sometimes instead a red-brown fluorescence was visible. The fluorescence of the theca cells and that of the granulosa cells did not

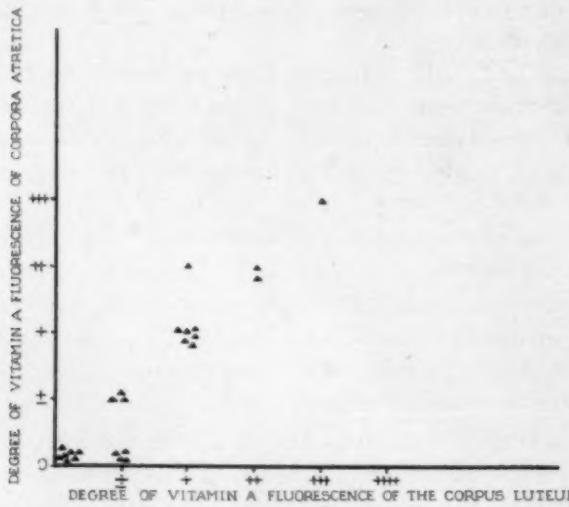

Fig. 4.—A comparison between the vitamin A fluorescence of the corpus luteum and that of the corpora atretica in the same ovary.

parallel each other. The fluorescence in each instance is apparently not related to that of other ovarian structures; e. g., it may be present early in the climacterium or in pregnancy when almost no vitamin A fluorescence is demonstrated in the rest of the ovary. From the few follicular cysts studied one gained the impression that their granulosa cells were richer in vitamin A fluorescence during the development of the early corpus luteum and that the theca cells were richer is concurrence with a mature or involuting corpus luteum.

Ovaries in the Climacterium.—After the climacterium vitamin A fluorescence was not encountered except in the granulosa cells of a follicular cyst. Early in the climacterium the scattered theca cells of the corpora atretica sometimes still showed the deep red-brown ultraviolet-

Source of Ovaries Studied with Average Distribution of the Vitamin A Fluorescence in Different Structures of the Ovary

Source of Ovaries Studied	Cases	Vitamin A Fluorescence in: Corpus Luteum — Granulosa	Corpus Luteum — Theca	Involuting Corpus Luteum	Stroma Around Involuting Corpus Luteum	Scattered Theca Cells	Corpus Atreticum	Stroma of Ovary	Primordial Follicle	Graafian Follicle
Premature and full term infants up to 5 months	3	.....	.....	.....	..	..	..	0	0	0
Children	5	.....	.....	.....	..	..	± to ++	+ to ++	0 to ±	± to +
Women at different phases of the menstrual cycle										
Early follicular phase	5	.....	.....	++ to +++	++	0 to +	++	.....	.....	±
Late follicular phase	1	.....	.....	+++	0	0	+	.....	.....	+
Early lutein phase	11	+	0 to ++	+++	+	0 to ±	+	.....	.....	.....
Late lutein phase	4	++	+ to ++	++	+	0 to ±	++	.....	.....	.....
Women with irregular menstruation	4	0 to +++	0 to ++	+						
Women in pregnancy										
Early pregnancy	7	±	0 to +++	.....	0 to ±	0	+	.....	.....	.....
Late pregnancy	4	0 to ±	±	.....	.....	0	±	.....	.....	.....
Women at the climacterium	9	.....	.....	.....	..	0	0	.....	.....	.....

0 indicates absence of vitamin A fluorescence; ±, traces; +, small amounts; ++, moderate amounts; +++, high amounts.

stabile fluorescence. Later on, the red-brown fluorescent cells disappeared, and only a few irregular small yellow or red fluorescent granules were seen in the blue fibrous stroma.

Ovaries Affected by Pathologic Conditions.—In cases of irregular menstruation the fluorescent details followed less clearly the outlined picture.

In the course of our study some abnormal structures were encountered. A corpus luteum cyst was rich in vitamin A fluorescence. A dermoid cyst was rich in lipoids but failed to show vitamin A fluorescence.

Uterus.—The uterine wall and endometrium were also studied in some cases. In several, vitamin A fluorescence was imparted by small lipoid droplets in all layers of the endometrium, particularly in the compact layer. The fluorescence was never bright and faded quickly. After this fading, a red-brown nonacetone-soluble pigment was observed, somewhat similar to that in the ovary. In other areas brown or yellow fluorescent pigment was observed immediately. We were unable to establish any parallelism between the vitamin A fluorescence in the endometrial stroma and that of the ovary.

COMMENT

The results obtained confirm those of our previous study, based on postmortem material.[3] In evaluating the significance of the vitamin A fluorescence in ovaries, the question arises as to the nature of the substance giving the characteristic fluorescence. Histochemical analysis in combination with animal experiments have brought conclusive evidence that the green fading fluorescence in sections of livers and adrenals is caused by vitamin A. The rat ovaries revealed a relatively slowly fading green vitamin A fluorescence in the interstitial cell cords and a quickly fading brighter one in fine lipoid droplets in the so-called corpora lutea.[4] This fluorescence was absent in vitamin A-deficient rats but reappeared when the animals were fed vitamin A or carotene. It was markedly increased in hypervitaminosis. These findings show conclusively that the fluorescence in the rat's ovary is due to vitamin A. In anatomic structure, however, the rat's ovary differs from the human ovary. Thus the findings in the rat's ovary can be applied less conclusively to the human ovary than is possible with the findings in the liver. Since, however, we do not know of any other substance in the human ovary which may cause the fluorescence, we believe that the characteristic fluorescence is actually due to vitamin A. That is supported by a chemical assay by Dr. I. Kraus. The Carr-Price reaction was strongly positive in several extracts of corpora lutea examined; the amount was higher than could be explained by the presence of carotene.

The presence of carotene in the ovary has been known for many years.[11] Probably the very dull, very slowly fading acetone-soluble fluorescence of the cytoplasm of the granulosa cells of the corpus luteum is due to carotene.

The faster fading vitamin A fluorescence shows characteristic variations with the menstrual cycle. It does not necessarily run parallel with the variation of the histologic lipoid distribution. The literature does not agree on the significance of the histologic lipoid distribution in the ovary and its relation to the endocrine function. Only a few see in the histologic fat reaction a sign of biologic activity[12]; most associate it with regression.[13] The chemical analysis for lipoids is considered far more significant.[14] Various staining reactions have been used in the morphologic demonstration of lipoids. The vitamin A fluorescence (independent of its physiologic significance) may be considered as a newly discovered morphologic characteristic of some of the ovarian lipoids. Not only does the fluorescence divide them into two groups, namely, those with and those without the fluorescence, but also the intensity and the type of fluorescence are characteristic. The study of the distribution of the vitamin A fluorescence in the ovary on a greater material and in various phases of ovarian function supports the previously made assumption [3] that the vitamin A fluorescence of the ovary is a morphologic sign of its endocrine activity.

The development of the corpus luteum is characterized by an increase in number, size and fluorescence of the lipoid droplets with the vitamin A fluorescence, as if the fluorescent material were being built up. In the granulosa cells of the mature corpus luteum the droplets are lined on the edge of the cells, and the morphologic picture agrees with the possibility that these fluorescent droplets are secreted into the capillaries. With beginning involution this picture of secretion changes into that of retention. The shrinking granulosa cell layer contains very large, strongly fluorescent drops. In addition, fine fluorescent lipoid droplets appear in the surrounding stroma, whereas the fat droplets in the fibrous center are free of the fluorescence. This picture Bartelmez has explained by the possibility that the involution is started by diminution of the blood supply to the corpus luteum with retention of the secretion within the

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12. Berberich, J., and Jaffé, R.: Ztschr. f. ang. Anat. **10**:1, 1925.

13. Marcotty, A.: Arch. f. Gynäk. **103**:63, 1914. Meyer, R.: ibid. **93**:355, 1911.

14. Kaufmann, C., and Raeth, K.: Arch. f. Gynäk. **130**:128, 1927. Traut, H. F.; Kuder, A., and Cadden, J. F.: Am. J. Obst. & Gynec. **38**:798, 1939. Weinhouse, S., and Brewer, J. I.: Proc. Inst. Med. Chicago, to be published.

granulosa cell layer, followed later by diffusion of the secretion into the tissue spaces of the surrounding stroma.[15] The final stage of involution is characterized by disappearance of the vitamin A fluorescence.

There is a characteristic difference in the fluorescence of granulosa and theca cells, not only in the corpus luteum but also in the follicular cysts or in the theca cells of the corpora atretica and their derivatives. In the granulosa cells the fluorescence is brighter, but faster fading; in the theca cells it is duller and slower fading and simulates thus somewhat the still dimmer and much more slowly fading carotene fluorescence. The theca cell fluorescence is often associated with double refraction. The same difference is seen in the rat's ovary and in ovarian tumors.

The corpora atretica which have been shown to be rich in estrogen and progesterone[16] may be rich in vitamin A fluorescence. The degree depends besides on the development of the follicle in which atresia started and the age of the corpus atreticum and on the degree of vitamin A fluorescence in the corpus luteum. This indicates that the vitamin A fluorescence of the corpus atreticum varies with the menstrual cycle. Even the dispersed theca cells after the dissolution of the corpora atretica (possibly the representatives of an interstitial gland) may still show some vitamin A fluorescence which disappears before the anisotropic lipoids and is (compensatorily?) stronger when the vitamin A fluorescence of the involuting corpus luteum is low.

This parallelism between vitamin A fluorescence and endocrine activity during the menstrual cycle agrees with the previously made assumption that the fluorescence is a morphologic sign of sex endocrine activity. It also agrees with findings on ovarian tumors[17]: endocrinously active tumors impart vitamin A fluorescence in contrast to the inactive forms like cystoma and cystadenocarcinoma. Whether the fluorescence indicates storage or formation of the hormones cannot be decided. Since both graafian follicles and fully developed corpora lutea impart the fluorescence, apparently the estrogen and the progesterone phase are associated with the fluorescence. One might correlate estrogen formation with the bright, quickly fading and progesterone formation with the duller, more slowly fading fluorescence.

The relation of vitamin A or the substance with the vitamin A fluorescence to the sex hormones is not clear. The nutritional status is without influence, as previously pointed out. That vitamin A fluorescence appears where the sterone hormones are formed, namely, in the adrenal cortex, in the interstitial cells of the testicle and in the ovary,

15. Bartelmez, G. W.: Personal communication to the authors.

16. Corner, G. W.: Physiol. Rev. **18**:154, 1938. Zondek, B., and Aschheim, S.: Arch. f. Gynäk. **127**:250, 1926.

17. Popper, H., and Ragins, A. B.: Arch. Path. **32**:258, 1941.

can be explained by identical affinity of some lipoids for vitamin A and sterone hormones. A direct relation of vitamin A to the sex hormones, however, cannot be excluded.

The absence of vitamin A fluorescence from the pregnant ovary is characteristic and may be even of diagnostic value. Not only the corpus luteum is poor in the fluorescence (except for some scattered lipoid droplets [18]), but also the corpora atretica and even the scattered theca cells, proliferated in pregnancy.[19] Possibly the high hormone secretion in pregnancy prevents storage of both the hormones and the lipoid carriers.

The picture of the child's ovary is surprising. According to the present view, it produces some estrogen and progesterone. In the first months of postnatal life, however, the maternal pituitary hormones may still exert some influence; possibly only after this time does vitamin A fluorescence appear in the child's ovary.

The nonfading red-brown fluorescent pigment in the corpora lutea and atretica, in the scattered theca cells, in the follicular cysts and even in the stroma is never seen in the granulosa cell layer; it is much less clearly seen in visible light as brown pigment and is apparently identical with the pigment described in theca lutein cells of the human atretic follicle [20] and in the regressing corpus luteum in Macaca mulatta.[21] It gives a sudan reaction, even in paraffin sections, since it is only partly removed by fat solvents and is insoluble in alcohol. It gives no iron reaction and does not derive from blood pigment. That it appears while the vitamin A fluorescence exists but increases with the disappearance of the fluorescence and is seen in greatest concentration in "burned out," apparently inactivated structures points to its being a "wear and tear" product. There is no evidence that it derives from vitamin A, since it is absent from other vitamin A-containing structures. A similar fluorescence is encountered where sterone hormones are or have been produced, as in the interstitial cells of the testicle or in the adrenal cortex (especially in the reticular layer); possibly it derives from the sterones or their carrier substances.

SUMMARY

Vitamin A fluorescence has been studied in ovaries of children and of women in the reproductive age (menstrual cycle and pregnancy) and in the climacterium in continuation of previous studies on the fluorescence microscopic picture of the ovary based on postmortem material.

18. Gillman, J., and Stein, H. B.: Surg., Gynec. & Obst. **72**:129, 1941.
19. Seitz, L.: Arch. f. Gynäk. **78**:203, 1906.
20. Adachi, S.: Ztschr. f. Geburtsh. u. Gynäk. **76**:125, 1914. Walthard, K. M.: ibid. **86**:74, 1923. Hohlweg and Dohrn.[ee]
21. Rossmann, J.: Anat. Rec. (supp.) **79**:53, 1941.

In this study, chiefly material obtained at operation has been utilized, and the vitamin A fluorescence has been correlated with the menstrual history and the histologic picture of the ovary and the endometrium. The fluorescence is seen in children's ovaries after the fifth month of postnatal life, in the stroma and the corpora atretica. In the reproductive age it is found in the corpora lutea and atretica, in follicular cysts and in certain areas of the stroma. It is absent at the climacterium.

The fluorescence is a morphologic characteristic of some of the ovarian lipoids. It is seen in typical distribution in theca and granulosa cells and undergoes characteristic variations during the menstrual cycle, especially during the development of the corpus luteum. The histologic picture of the mature corpus luteum suggests that the fluorescent material is being secreted into the blood capillaries; in pregnancy the fluorescence is nearly absent, possibly due to exhausting secretion.

In various structures of the ovary a brown fluorescent substance is encountered, which is probably a "wear and tear" product.

The typical variations of the vitamin A fluorescence in amount and distribution indicate a relation to sex hormone production or storage. It is apparently superior to other lipoid reactions as a morphologic sign of sex endocrine activity, independent of its physiologic significance. It is not established whether vitamin A or the fluorescent substance is chemically or biologically related to the sex hormones. The parallel appearance is possibly explained by similar affinity of vitamin A and the sterone sex hormones to the same lipoid carrier substance.

COMPARISON OF PATHOLOGIC OBSERVATIONS IN WEIL'S DISEASE AND IN YELLOW FEVER

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In this vicinity, not far distant from the tropics, epidemics of yellow fever have been recorded in past decades. As a fatal case of Weil's disease recently occurred in which a necropsy was made, it seemed of interest to report the pathologic observations as compared with those on material available from long past local occurrences of yellow fever. Especially did this seem indicated as it is now generally accepted that Noguchi [1] in his primary work on the cause of supposed yellow fever had in reality dealt with Weil's disease. Furthermore, it has been recently stated [2] that Weil's disease is probably on the increase in the United States. It appears, therefore, of value to record established occurrences of this infection. For careful reviews of the general literature on this subject the reader is referred to that by Ashe, Pratt-Thomas and Kumpe [3] and that by Jeghers, Houghton and Foley.[4] According to the former writers, while reports of cases in Europe are numerous, the total in America is 67. To these might be added 4 cases of recent occurrence in this vicinity reported by Wilen, Snavely and Bruno [5] and 2 others mentioned by them, another case by Durand,[6] 4 cases by Reid and Holt [7] in Virginia, 3 cases by White and Prevost [8] and 6 cases by Rathbun and Waghelstein,[9] making a total of 87. Although Weil [10] is credited with the first replete description of this disease, in 1886, this was not the first reference to the condition.

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1. Noguchi, H.: J. Exper. Med. **19**:547, 1919.
2. Weil's Disease, editorial, J. A. M. A. **117**:1266, 1941. Spirochetal Jaundice, editorial, ibid. **109**:1128, 1937.
3. Ashe, W. T.; Pratt-Thomas, H. R., and Kumpe, C. W.: Medicine **20**:145, 1941.
4. Jeghers, H. J.; Houghton, J. D., and Foley, J. A.: Arch. Path. **20**:445, 1935.
5. Wilen, C. J.; Snavely, J. R., and Bruno, T. E.: New Orleans M. & S. J. **94**:338, 1942.
6. Durand, J. A.: New Orleans M. & S. J. **94**:341, 1942.
7. Reid, J. D., and Holt, R. A.: Virginia M. Monthly **68**:571, 1941.
8. White, J. J., and Prevost, J. V.: Ann. Int. Med. **15**:207, 1941.
9. Rathbun, H. K., and Waghelstein, J. M.: Ann. Int. Med. **15**:395, 1941.
10. Weil, H. A.: Deutsches Arch. f. klin. Med. **39**:209, 1886.

Some state that the entity was probably recognized by Hippocrates. The historical aspect has been studied by Bates [11] and Dawson, Hume and Bedson.[12] The discovery by Inado and co-workers [13] in 1914 that the causative factor was a spirochete served to establish the specific identity of this disease. Noguchi [14] classified the micro-organism and named it Leptospira icterohaemorrhagica. About that time, which was during the last World War, various writers [15] made contributions on Weil's disease that are significant. These include numerous cases in the armies.

Of the 4 cases of Weil's disease recently reported in this community by Wilen, Snavely and Bruno,[5] the one concerned here was the only instance in which the disease proved fatal, thereby permitting pathologic study.

REPORT OF A FATAL CASE OF WEIL'S DISEASE

A digest of the clinical aspect of the disease in this case reveals that about five days prior to admission to the hospital the patient, a white man 39 years of age, noted a sudden onset of aching pains in both forearms. He had chills and fever that day and the next day. Weakness of the muscles ensued, and aching became generalized, including headache. On the third day of his illness, he began to vomit. The vomitus was watery and dark green. Tinnitus aurium was present for four days. There was a slight nonproductive cough. Anorexia and dysphagia developed, with a resulting loss of 10 pounds (4.5 Kg.) during five days. The patient was constipated; no melena was noted. The urine was of a dark color and the output scanty.

It is of interest that about ten years previously he had had two attacks of jaundice during the same year. There had been, however, no muscle pains or other phenomena suggestive of Weil's disease with those two episodes. He was a dairy hand.

On admission the temperature was 97.4 F., the pulse rate 100, the respiratory rate 30 and the blood pressure 75 systolic and 60 diastolic. He appeared severely ill. The skin was icteric, hot and dry. He was well oriented but very weak. The pupils were small, equal and active. The scleras were yellow and injected. The pharynx was reddened, and the buccal mucous membrane was icteric. There was general abdominal rigidity with tenderness in the right upper quadrant. The reflexes were symmetric and physiologic. The erythrocyte count was 2,900,000 and the leukocyte count 15,000 with 97 per cent polymorphonuclear leukocytes, 1 per cent lymphocytes and 2 per cent monocytes. The urine contained much albumin, a large number of red blood cells, leukocytes and hyaline and finely granular casts. Chemical examination of the blood showed urea 16.1 mg. and dextrose 143 mg. per hundred cubic centimeters. The icterus index was 110,

11. Bates, J. E.: Canad. M. A. J. **16**:1466, 1926.

12. Dawson, B.; Hume, W. E., and Bedson, S. P.: Brit. M. J. **2**:345, 1917.

13. Inado, R.; Ido, Y.; Hoki, R.; Kaneko, R., and Ito, H.: J. Exper. Med. **23**:377, 1916.

14. Noguchi, H.: J. Exper. Med. **27**:575, 1918.

15. (*a*) Dawson, B., and Hume, W. E.: Quart. J. Med. **10**:90, 1916-1917. (*b*) Stokes, A.; Ryle, J. A., and Tytler, W. H.: Lancet **1**:142, 1917. (*c*) Dawson and others.[12]

and the van den Bergh reaction (direct) indicated a bilirubin content of 21.6 mg. per hundred cubic centimeters of serum. The Wassermann reaction and the blood culture were negative, and a roentgenogram of the chest revealed no change.

After admission, the patient's temperature began to rise, reaching 102 F. The next day he appeared much worse and delirious. Rales were heard at the bases of both lungs. A pericardial friction rub was also heard at this time. He gradually grew weaker and died two days later. Just prior to death he had a momentary convulsion. At all times his pulse rate seemed to correspond to his temperature curve. The clinical diagnosis was: Weil's disease; bronchopneumonia; hepatitis; pericarditis; myocarditis.

Necropsy.—The body was that of a well developed, moderately well nourished white man about 40 years of age. The skin showed marked jaundice and numerous petechial areas, most notable over the chest. The eyes presented yellow conjunctivas and scleras. Subconjunctival hemorrhages were present in both eyes but especially in the left. The pupils were equal, measuring 5 mm. A few small, easily movable inguinal and axillary lymph nodes were palpable. The liver's edge could be felt about 7 cm. below the costal margin.

When the abdomen was opened, the subcutaneous tissue had a yellow color. Numerous petechial spots and ecchymotic areas were seen scattered diffusely over the parietal peritoneum.

The lungs were voluminous and filled the pleural cavities. On the right side, extending from the apex to the base, were numerous firm fibrous adhesions. These were particularly dense overlying the diaphragm. The parietal pleura on the right, after the adherent visceral pleura had been detached, appeared rather purple and hemorrhagic.

The right lung weighed 800 Gm. and the left 925 Gm. The external surfaces had a mottled brown appearance. They were of diminishd consistency throughout, but there was complete absence of crepitation in most of the upper lobe of the left lung. On cut section the lower lobe of the right and both lobes of the left lung were quite firm and brown. In the bronchial tree a small amount of frothy fluid was present. The tracheobronchial lymph nodes appeared slightly enlarged. Some were dark red on cut section, while others were gray to black.

The pericardial cavity contained about 10 cc. of clear fluid. Some fibrinous exudate was seen on the epicardium in the region of the right auricle. There were also ecchymoses and petechiae on both the visceral and the parietal layer of the pericardium. The heart weighed 350 Gm. It was brown and firm. On external examination the right side of the heart appeared to be slightly dilated. When the heart was opened, scattered petechial hemorrhages were seen in the endocardium.

The spleen weighed 175 Gm. Its external surface was gray and wrinkled. The cut section was purple, and the malpighian corpuscles were prominent. A considerable amount of fibrous tissue was present throughout, rendering the organ somewhat firm.

The liver weighed 1,825 Gm. Its external surface was smooth and light brown. It was quite firm throughout.

The gallbladder was slightly distended, containing about 30 cc. of dark red to brown viscid bile. The mucosa was red. No calculi were present, nor was there any evidence of obstruction to the bile passages.

The pancreas weighed 100 Gm. It was yellow and presented the normal lobulated appearance.

The esophagus had a smooth white lining, and no varices were noted. The stomach showed prominent rugae, and the mucous membrane was dark red.

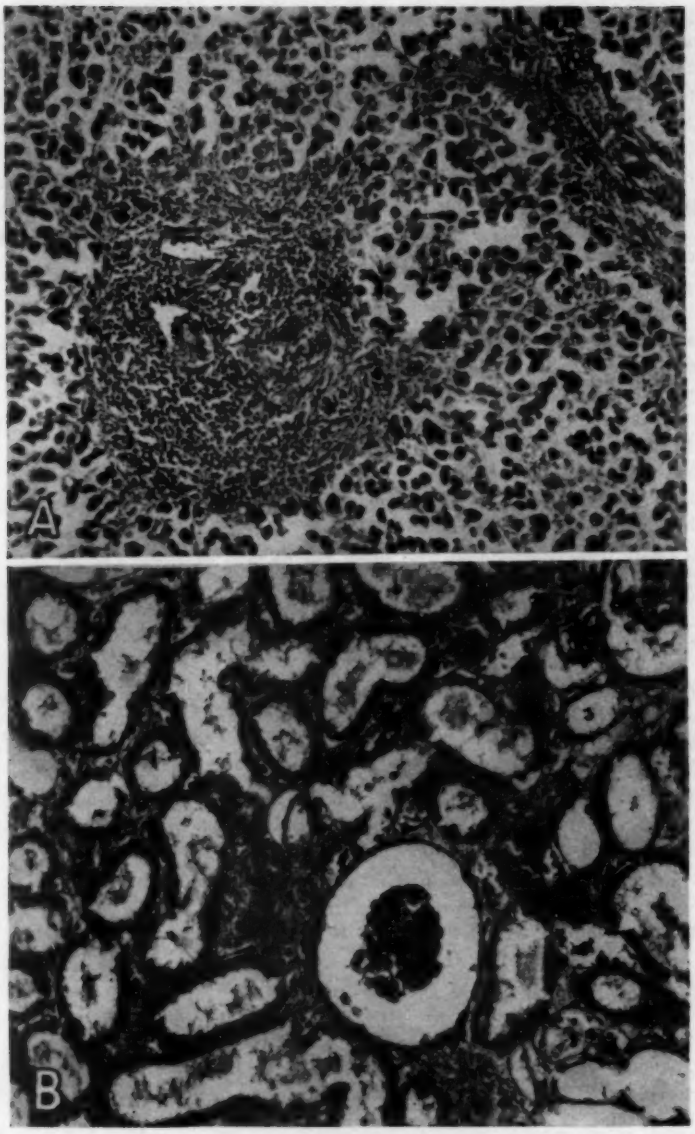

Fig. 1.—*A*, photomicrograph of liver in Weil's disease; × 150. It reveals the disorganization of the lobular cords of cells into separate cellular units. Most of these contain one or more nuclei, many of which show pyknosis. Note the lymphoid cellular accumulations in the portal tracts. *B*, photomicrograph of kidney in Weil's disease; × 150. Degenerative changes are present in the epithelial lining of the tubules, and much granular detritus can be seen in the lumens. Some of the tubules are dilated and have almost completely lost their epithelial lining. The renal corpuscles show marked atrophy of the glomerular tufts. Scattered lymphoid cells are seen in the interstitial tissue.

There was present about 100 cc. of yellow semisolid material, partly composed of undigested food, with no evidence of fresh or altered blood. The lining of the duodenum was also red and presented prominent rugae. The duodenal changes did not appear accentuated at the ampulla of Vater. The jejunum, ileum and upper portion of the colon contained a considerable amount of dark red semisolid material. Their walls were hemorrhagic in appearance. Just beneath the serosa of various portions of the intestines were petechiae.

The adrenals appeared normal in size. They showed no evidence of hemorrhage. The cortex and medulla of each were well differentiated.

The right kidney weighed 210 Gm. and the left 240 Gm. Both were light brown, smooth in contour and fairly firm. The capsules stripped with ease, leaving smooth light brown cortical surfaces. On cut sections the cortices and medullae were well defined and presented no definite prominence of the glomerular or of the tubular structures. The lining of the pelves showed small petechial areas on its surface.

The urinary bladder contained approximately 40 cc. of yellow urine. The mucosa presented a trabeculated appearance. The prostate appeared normal externally and on section, as did the testes.

Microscopic Study.—In sections of the heart the epicardium appeared, for the greater part, normal; in some areas there was fibrinous exudate on the surface; congestion of vessels with collections of lymphocytes were seen in the underlying connective tissue. The myocardium was normal, with a moderate amount of fibrous tissue stroma between the myocardial fibers. In some areas were free red blood cells.

In sections of the lungs the pleural surface was irregular because of the presence of numerous free red blood cells, some fibrin and a few fibroblasts and lymphocytes. Most of the alveoli were packed with red blood cells together with many "heart failure" cells, a few round cells and a few polymorphonuclear leukocytes. The "heart failure" cells contained "smudges" of light brown pigment together with varying-sized granules of dark brown pigment.

In the spleen there was some increase of the reticular supportive tissue throughout. There were many round cells and reticulocytes scattered diffusely throughout the splenic pulp. Many of the latter contained small amounts of light to dark brown pigment in clumps and granules. The malpighian corpuscles were not sharply demarcated and appeared rather small.

The liver revealed considerable disorganization of the arrangement of the liver cells. Instead of appearing as continuous cords of cells, they occurred individually or as two or three cells together, thus showing marked dissociation (fig. 1 *A*). They were separated by delicate strands of connective tissue. In some areas the spaces between the liver cells were greatly widened. The individual liver cells showed rather granular cytoplasm, which had a slightly basophilic hue. There were only occasional light to dark brown granules in the cytoplasm. The nuclei were round and fairly regular in size, and contained one to three nucleoli and a sparse amount of diffuse small chromatin granules. No definite karyolysis or karyorrhexis was seen, but distinct pyknosis was present. The portal spaces and the divisions of Glisson's capsule were thickened, because of the presence of an increased amount of fibrous tissue and numerous fairly crowded collections of lymphocytes. No bile pigment was seen in the bile capillaries or ducts. The Kupffer cells did not appear to be increased in number nor did they contain pigment.

The kidneys showed a rather diffuse infiltration of collections of round cells. These were more numerous in the cortical portion and especially near the glo-

meruli. There was an increased amount of connective tissue in both the cortex and the medulla but particularly in the region of the renal corpuscles and between the convoluted tubules. Many dilated endothelial-lined spaces filled with blood were seen in the medulla and to a less extent in the cortex. The glomerular tufts were small, leaving large capsular spaces, which occasionally contained serum and a moderate number of red blood cells. The tubular epithelium was granular. In some areas the tubules were distinctly dilated. Many contained a small amount of amorphous pink-staining granules. The borders between the cells lining the tubules were poorly defined (fig. 1 *B*).

Sections of the marrow showed a moderate number of nucleated erythrocytes together with cells of the myeloblastic and lymphoblastic series.

The Levaditi stain for spirochetes was done on sections of the various organs. All, after careful and prolonged examination, were found not to contain spirochetes, excepting sections of the kidneys. These revealed many black-staining tightly coiled spiral micro-organisms in the epithelium of the convoluted tubules and in the stroma between these structures. The micro-organisms varied in length from approximately 6 to 12 microns. They contained six to eight tight coils and a few gentle undulations. Some showed hooked terminations at either one or both ends (fig. 2).

The histologic diagnosis was Weil's disease. Blood from the heart was examined at the National Institute of Health for agglutination of L. icterohaemorrhagiae, and a positive result was obtained up to a dilution of 1:500. About 1 cc. of serum from the heart's blood was injected into each of 3 white rats. They showed no clinical evidence of the development of an illness. Inoculation of suspensions of the kidneys of these rats into guinea-pigs gave negative results, as did also Levaditi stains of these organs.

There are two explanations for the failure to recover the causative micro-organism: First, the amount of cardiac blood available for inoculation was smaller than that usually recommended (5 to 6 cc. of whole blood); second, the spirochetes doubtlessly were scarce in the patient's blood, as suggested by the early positive agglutination reaction.

COMPARISON WITH YELLOW FEVER

The similarity between Weil's disease and yellow fever in both clinical and pathologic features is strikingly demonstrated through the etiologic investigations of Noguchi [1] on supposed yellow fever. Having isolated a pathogenic spirochete, he believed he had solved the problem. Eventualities have shown that the cases supplied to him most likely were instances of Weil's disease or at least "spirochetal jaundice," and not of yellow fever. Concerning his cases, he stated: "The clinical and pathological features of yellow fever prevalent in Guayaquil conform with those described by other investigators of this disease as it has occurred elsewhere, both epidemically and endemically." Thus those very familiar with yellow fever and Weil's disease at that time erred in their differentiation of the two conditions.

Stock tissues obtained locally several decades ago in 2 cases diagnosed clinically and pathologically as yellow fever were available to me. They afforded the means for a comparison of the histologic changes in yellow fever with those in the current case of Weil's disease. Sections of this material were stained by both the eosin-hematoxylin and the Levaditi method.

Fig. 2.—Photomicrograph of kidney (Levaditi stain) showing Leptospira icterohaemorrhagiae in tubules above and in the interstitial structure at the midportion; × 500. Insert shows higher magnification of a typical spirochete in the same organ; × 1,000.

In general, the most marked microscopic changes were noted in the liver and the kidney. In 1 instance the mucosa of the stomach revealed areas of necrosis and some hemorrhagic extravasation. The spleen in this same case presented small areas of hemorrhagic extravasation. The sinuses were dilated and contained shadow erythrocytes, indicative

of hemolysis. Much blood pigment, both free and phagocytosed, was noted in various portions of the structure, especially in the cords of the splenic pulp. The splenic corpuscles presented nothing noteworthy.

In the liver in both cases very marked degenerative and necrotic changes were seen, with comparatively little normal structure present.

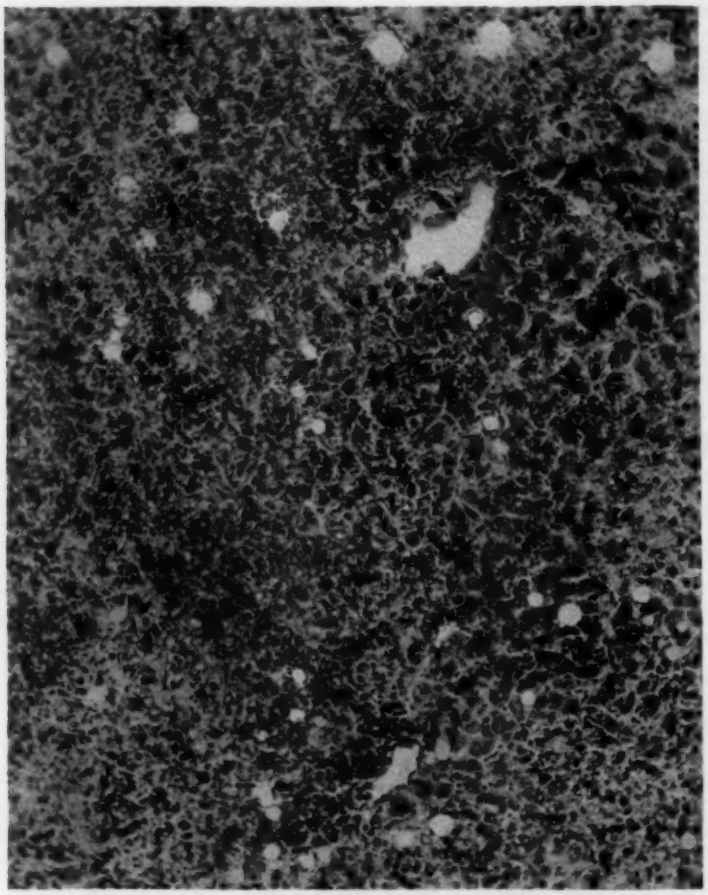

Fig. 3.—Photomicrograph of liver in yellow fever; × 150. The lobular hepatic cords are more or less confluent. Degenerative changes of the fatty, granular and necrotic types are seen. The retrograde changes are present in the mid-zonal and central areas. Some cells at the peripheral portions of lobules are less involved.

The degenerative changes were only in small part granular and fatty, the more extensive portion being hydropic (fig. 3). The cellular necrosis was scattered focally and in some instances presented highly eosinophilic stained areas that had been described as Councilman lesions.

No distinctive midzonal localization could be noted for this lesion, as it was so generally disseminated. No cellular inclusions were observed except such fragmentary particles as are usually present in extensive hepatic necrosis. Scattered blood and bile pigments were noted, the former especially in the sinusoids and the latter in the hepatic cells. In the kidneys, the tubules of the cortical portion, especially those of the convoluted type, revealed marked granular degeneration with granular accumulations in the lumens. Some of the cells presented karyolysis of the nuclei, and others were pyknotic, indicative of cellular necrosis. Fragmentation and desquamation of the cellular lining were occasionally noted. In the majority of the renal corpuscles there were accumulations of serum in the subcapsular spaces and varying degrees of atrophy of the glomerular tufts. A few of the spaces contained red blood corpuscles. In the medullary portion varying degrees of parenchymatous change were noted in the tubular epithelium, and many of the involved tubules were found to contain casts. There was marked congestion of the blood vessels, particularly of the smaller ones, in the intertubular spaces. It was noted in some instances that phantom red cells, indicative of hemolysis, were present. Here and there in the vessels, the epithelium and the glomerular tufts, scattered particles of blood pigment were seen.

COMMENT

The gross and microscopic changes described herein for Weil's disease conform in greater part to those found by other observers.[16] As has been noted by others, these changes are not specific for this disease but are such as might be encountered in other conditions in which there is infection with toxic manifestations and hepatic damage. Grossly, the main findings are jaundice and a hemorrhagic tendency as indicated by petechiae in the skin and mucous membranes, the organs revealing no characteristic changes. It is to be noted that splenomegaly is not present in this case, which is in accord with the general observations concerning the spleen in Weil's disease, although at variance with Weil's first description. Microscopically, changes are seen chiefly in the lungs, the liver and the kidneys. They consist mainly of hemorrhage in the lungs and varying degrees of degenerative change in the liver cells and in the proximal convoluted tubules of the kidneys, such as were noted in the present instance. Additional findings are often mentioned for the skeletal muscles, such as vacuolation, swelling,

16. (*a*) Gaines, Q. R., and Johnson, R. P.: Arch. Int. Med. **60**:817, 1937. (*b*) Ball, H. A.: Am. J. Clin. Path. **3**:283, 1933. (*c*) Hayman, J. M., Jr., and Lynch, T. B.: Am. J. M. Sc. **173**:80, 1927. (*d*) Cushing, E. H.: J. A. M. A. **89**:1041, 1927. (*e*) Dragert, E.: Virchows Arch. f. path. Anat. **292**:452, 1934. (*f*) McNee, J. W.: J. Path. & Bact. **23**:342, 1919. Jeghers and others.[4] Dawson and others.[12] Bates.[11] Dawson and Hume.[15a]

hyalinization and infiltration by polymorphonuclear leukocytes and plasma cells.[3] Similar changes are sometimes found in cardiac muscle.

An excellent discussion of the pathogenesis and the relation of the clinical findings to the lesions is presented by Ashe, Pratt-Thomas and Kumpe,[3] in which the mechanism of the jaundice, the hemorrhagic tendency and the muscular aching and tenderness are thoroughly discussed. It seems logical that the jaundice is due in large part to true hepatitis, since the finding of hepatic damage is so constant.[17] Dawson and Hume [15a] placed emphasis on the finding of inflammatory changes in the duodenum with swelling of the papilla of Vater, causing biliary obstruction. This is not always seen, however. Jaundice may thus be contributed to by obstruction of the biliary tract (extrahepatic or intrahepatic) and by hemolysis. Capillary damage seems to underlie the hemorrhagic tendency. The pathologic changes described for skeletal muscle give a basis for the muscular aching and tenderness noted clinically.

The spirochetes were found only in sections of the kidneys in this case. This is the organ in which they are most frequently encountered. Ashe, Pratt-Thomas and Kumpe [3] gave the following order of frequency of occurrence in the various organs: kidneys, liver, adrenals, myocardium, intestine, appendix and pancreas.

In yellow fever the retrograde cellular changes present in the tissues are usually more extensive than those noted in Weil's disease. While it appears that the virus of yellow fever presents in general a more devastating effect, particularly on the liver and the kidneys, variations and an approach to similarity do occur. Hence the changes noted in yellow fever, while quite constant, may vary in degree, and an absolute diagnosis on such a basis is to be questioned. Certain suggestive features of bright eosinophilic staining foci of necrosis in the liver, as well as cellular inclusions, may have their analogue in focal necrosis due to other causes, such as that in eclampsia, typhoid fever and the varying degrees of acute yellow atrophy.

In the present study of 2 cases clinically and pathologically diagnosed as yellow fever, the absence of leptospiras after careful and prolonged search of the organ sections appears to identify the disease by the process of exclusion.

SUMMARY

The pathologic changes in a case of Weil's disease are described and compared with those in 2 cases of yellow fever.

The salient discernible difference noted for these two diseases is that the amount of tissue injury in both the liver and the kidneys is in

17. Jeghers and others.[4] McNee.[16f]

general more extensive in yellow fever than in Weil's disease. The difference is known to be variable, however, and therefore it should not be considered as pathognomonic. A definite differentiation seems dependent on the presence or the absence of Leptospira icterohaemorrhagiae in Levaditi-stained sections.

It is pointed out that either Weil's disease is on the increase in this country or the recognition of the disease is becoming better established.

TUMORS OF THE STRIATOTHALAMIC AND RELATED REGIONS

THEIR PROBABLE SOURCE OF ORIGIN AND MORE COMMON FORMS

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The modern classification of primary neuroectodermal tumors of the nervous system and particularly of those of the brain is based on the hypothesis that these tumors take origin from unripe neuroectodermal derivatives and that in their variable histologic features they duplicate one or another phase in the histogenesis of these derivatives. It is thus assumed that in the developing brain some immature cell groups are left by the wayside as embryonal rests, which at one time or another in the life of the mature brain are recalled to new cellular activity, resulting in the formation of a neoplasm. This concept is well supported by the observation that in the developing brain there are certain zones in which critical developmental processes take place at a relatively late stage in the maturation of this organ and as such may harbor embryonal rests, likely to develop into neoplasms.

Attention has been called by one of us (Globus [1]) to such a zone, the striatothalamic region, as a common site for the development of one form of tumor, the so-called spongioneuroblastoma. This observation has found added support in the study of recently accumulated material and has prompted further investigations along these lines. It was with this in mind that it was found desirable to survey the embryologically significant striatothalamic junction, to make a careful search for the sources of embryonal rests in that region and to review the histologic and biologic character of tumors encountered in that and related regions. It was felt that the observations growing out of such a study might throw further light on the probable origin of a great number of primary neuroectodermal tumors of the brain.

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From the Laboratories of the Neurological and the Neurosurgical Services of the Mount Sinai Hospital, New York, and the Department of Anatomy of the Woman's Medical College of Pennsylvania, Philadelphia.

1. Globus, J. H.: Am. J. Cancer **32**:163, 1938.

EMBRYOLOGY OF THE CORPUS STRIATUM AND THE STRIATOTHALAMIC JUNCTION (SULCUS TERMINALIS)

The corpus striatum takes its origin from a thickening of the ventral portion of the telencephalic wall. In the human brain this thickening becomes conspicuous at first about the seventh week of intrauterine life, as seen in a fetus approximately 16 mm. in crown-rump length (fig. 1 *A*). This thickened region in the ventrolateral wall of the telencephalon subsequently gives rise to two prominences (fig. 1 *B*), which are known respectively as the medial and the lateral ganglionic ridge of

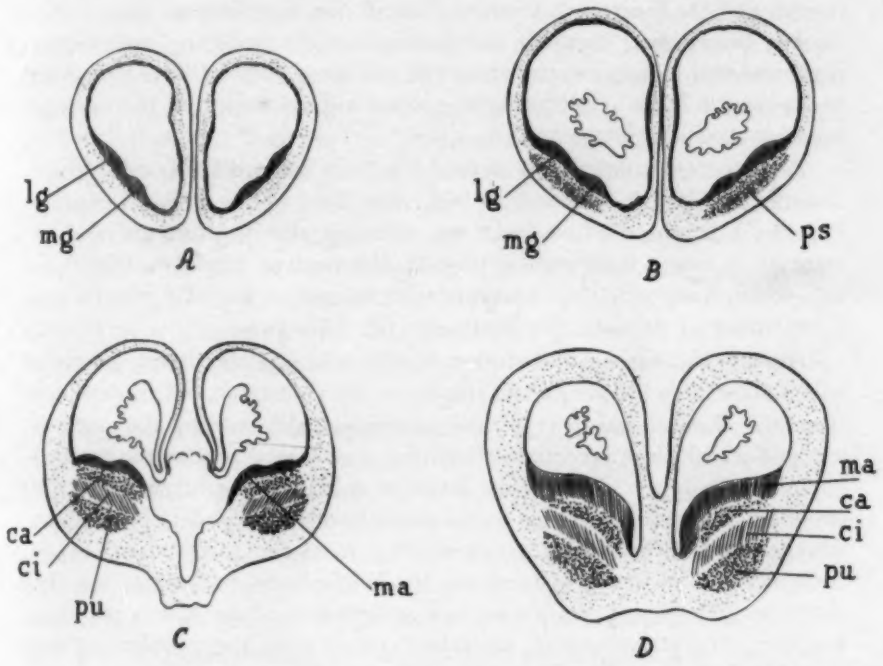

Fig. 1.—*A* represents the corpus striatum in the human fetus at 7 weeks, *B* at 8 weeks, *C* at 10 weeks (beginning of internal capsule formation) and *D* at 13 weeks; *ca* indicates the primordium of the nucleus caudatus, *ci* the internal capsule, *lg* the lateral ganglionic ridge, *ma* the matrix of the striatum, *mg* the medial ganglionic ridge, *ps* the primordium of the corpus striatum and *pu* the putamen. (Drawings after Hochstetter.[3])

His.[2] The development of these structures during the later critical period, between the seventh and the thirteenth week of intrauterine life,

2. His, W.: Abhandl. d. math.-phys. Cl. d. k. sächs. Gesellsch. d. Wissensch. **15**:675, 1889.

was well illustrated in a monograph by Hochstetter,[3] while their comparative anatomy, their phylogenetic and ontogenetic significance and especially their participation in the formation of the basal ganglions and the cerebral cortex were discussed at greater length by Kuhlenbeck.[4] Here it will suffice to point out that the lateral ganglionic ridge corresponds to the vertebrate longitudinal telencephalic zone D1 while the medial ganglionic ridge corresponds to the telencephalic zones B1 and B2 (Kuhlenbeck [5]).

In mammals these two ganglionic ridges gradually merge into one continuous thickening. In man this process takes place between the twelfth and the fourteenth week of intrauterine development (fig. 1 *D*). Shortly before their merging, the matrix of these two ganglionic ridges is represented by a common primordial cell mass (fig. 1 *B*), homologous to the mantle layer, constituting the common primordium of the nucleus caudatus and the putamen.

At about the ninth week of fetal life, at a stage corresponding to a crown-rump length of about 30 mm., the fibers of the internal capsule (fig. 1 *C*) begin to make their way through this primordium and to separate it into a dorsomedial portion, the nucleus caudatus (fig. 1 *C*, *ca*), which maintains its connection with the periventricular matrix and a ventrolateral portion, the putamen (fig. 1 *C*, *pu*).

Among the other subdivisions of the telencephalic basal ganglion which arise from the ganglionic ridges are the claustrum, which develops from the dorsolateral part of the ganglionic ridges, and the nucleus amygdalae, which differentiates from the most caudal part of the merged ganglionic ridges. It is still doubtful whether the matrix of the ganglionic ridges contributes to the formation of the globus pallidus or whether this nuclear mass arises entirely from the rostrolateral hypothalamic mass of neuroblasts within the hemispheric stalk adjoining the putamen. The claustrum, the nucleus amygdalae and the globus pallidus, however, are structures of secondary interest in the problem under consideration.

Rostrad to the interventricular foramen each lateral ventricle contains within its walls the head of the caudate nucleus laterally and the septum pellucidum medially. Caudad to the interventricular foramen, in the region corresponding to the body and tail of the caudate nucleus the topographic relationships become complicated by the striatothalamic junction. In this broad zone of junction, through which pass numerous

3. Hochstetter, F.: Beiträge zur Entwicklungsgeschichte des menschlichen Gehirns, Leipzig, Franz Deuticke, 1919, pt. 1.

4. Kuhlenbeck, H.: Vorlesungen über das Zentralnervensystem der Wirbeltiere, Jena, Gustav Fischer, 1927.

5. Kuhlenbeck, H.: Anat. Anz. **67**:1, 1929.

fiber systems, such as the internal capsule and the various thalamic stalks, the striatum adjoins the thalamus. The line of striatothalamic junction is marked by a groove, the sulcus terminalis.

The analysis and understanding of the developmental events in the zone of the striatothalamic junction have met with considerable difficulty. A number of authors (Mihalkovic,[6] Kölliker [7] and His [2]) assumed the occurrence of a secondary fusion between the lateral surface of the diencephalon and that part of the hemispheric wall which corresponds to the striatum. This view is still widely accepted and often quoted in the neurologic literature. On the other hand, careful studies of the development of the striatothalamic junction by Schwalbe,[8] Goldstein,[9] Hochstetter [3] and Kuhlenbeck [5] have shown that such a secondary fusion does not occur and that the broad zone of continuity between the striatum and the diencephalon derives from the thickening of a primary zone of transition between the telencephalon and the diencephalon, resulting in the formation of the so-called hemispheric stalk (fig. 2).

In the human brain, at about the sixth week of intrauterine life (11 mm. crown-rump length) the hemispheric vesicle is demarcated from the rostral end of the diencephalic ventricle by a low ridge in the floor of the primitive interventricular foramen. This is the torus hemisphaericus, which represents the rostral part of the hemispheric stalk and corresponds on the external surface to the telencephalodiencephalic groove (fig. 2).

The right and left telencephalodiencephalic grooves converge rostrad in the direction of the lamina terminalis to join and form, according to Kupffer,[10] the torus transversus. The latter is the thickened portion of the lamina terminalis in which develops the anterior commissure as a part of the commissural plate. As the telencephalic vesicles extend caudally, the hemispheric stalk continues to retain its primary connection with the lateral wall of the diencephalon. On its ventral aspect the hemispheric stalk contains the ventrocaudal portion of the telencephalodiencephalic sulcus, while dorsolaterally it includes a groove formed by the floor of the caudal part of the lateral ventricle (fig. 2 *C*). This

6. Mihalkovic, V. V.: Entwicklungsgeschichte des Gehirns, Leipzig, Wilhelm Engelmann, 1877.

7. Kölliker, A. V.: Entwicklungsgeschichte des Menschen und der höheren Tiere, Leipzig, Wilhelm Engelmann, 1879.

8. Schwalbe, G.: Sitzungsb. d. Jenaisch. Gesellsch. f. Med. u. Naturw., 1880, p. 20.

9. Goldstein, K.: Arch. f. Anat. u. Entwcklngsgesch., 1903, p. 29.

10. von Kupffer, K.: Die Morphogenie des Zentralnervensystems, in Hertwig, O.: Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere, Jena, Gustav Fischer, 1905, vol. 2, pt. 3, pp. 241-272.

groove is bounded laterally by the ventrolateral portion of the telencephalic wall in which the ganglionic ridges develop, and medially by the portion of the thin lamina epithelialis of the roof plate of the telencephalon ventrad to the choroid fissure. This portion of the epithelial plate is adjacent to the lateral diencephalic wall with which it fuses subsequently to become the lamina affixa. The fused ganglionic ridge which develops here into the body and tail of the caudate nucleus thus adjoins the lateral wall of the thalamus covered by the lamina affixa, and the intraventricular groove between the caudate nucleus and the thalamic surface constitutes the sulcus terminalis (figs. 2 and 3).

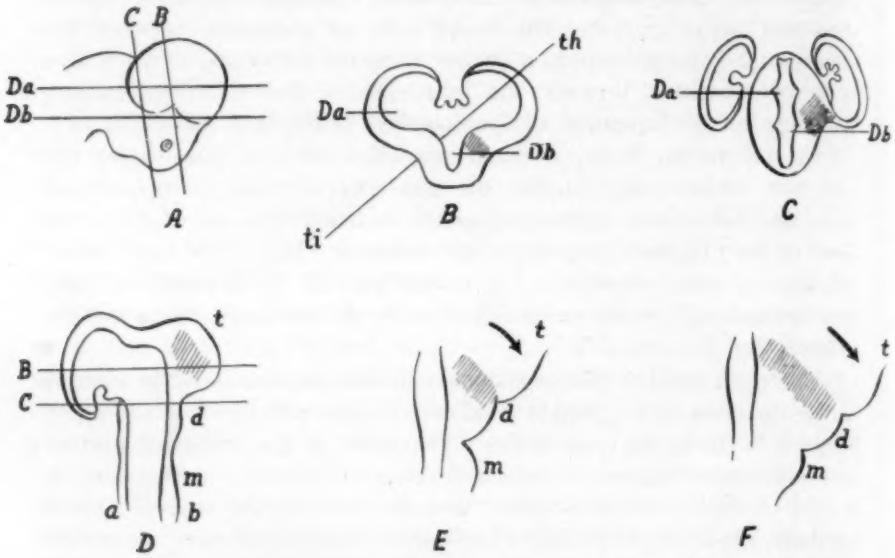

Fig. 2.—Diagram illustrating the development of the hemispheric stalk in a human fetus of 11 mm. crown-rump length (after Kuhlenbeck [4]): *A*, vertical section; *B* and *C*, horizontal sections. The lines *Da* and *Db* show the planes of the sections in *B* and *C*. Drawings *D*, *E* and *F* show the fanlike widening of the hemispheric stalk; *d* indicates the diencephalon, *m* the mesencephalon, *t* the telencephalon, *th* the torus hemisphaericus, *ti* the sulcus diencephalotelencephalicus and the shaded areas the hemispheric stalk.

The subsequent development of the brain is marked by a growth of the hemispheres which exceeds that of the diencephalon. The lateral surface of the diencephalon dorsal to the hemispheric stalk, the part which originally projected upward between the hemispheres, flattens and becomes a dorsal instead of a lateral surface (fig. 3). The hemispheric stalk in the meantime broadens by means of a fanlike expansion, with its base becoming oriented in an anterolateral direction. The lateral surface of the diencephalon, the part caudal to the hemispheric

stalk, rotates gradually to face caudolaterad and finally becomes a caudal instead of a lateral surface (fig. 2). The assumed disappearance of the lateral surfaces of the embryonal diencephalon, a process which puzzled many investigators and prompted the hypothesis of a secondary telodiencephalic fusion of these surfaces, is therefore only apparent. The lateral surfaces remain as such, but their orientation is altered by the processes of development resulting in the thickening of the hemispheric stalk and in the overgrowth and overexpansion of the cerebral hemispheres.

With the formation of the temporal lobes and their poles, the sulcus terminalis accompanied by its neighboring structures, the tail of the

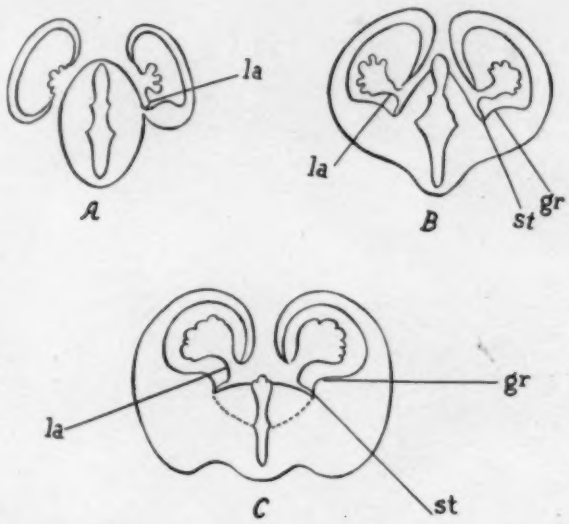

Fig. 3.—Three stages in telencephalodiencephalic development (modified after Hochstetter [8]). *A* represents the human fetus at 7 weeks, *B* the human fetus at 8 weeks and *C* the human fetus at 13 weeks; *gr* indicates the ganglionic ridge, *la* the lamina affixa and *st* the sulcus terminalis.

caudate nucleus and the lamina affixa, curves around the hemispheric stalk and extends as far as the tip of the descending horn of the lateral ventricle.

This mode of development reveals the fact that the histogenetic processes which are involved in the formation of the corpus striatum take place along the entire extent of the sulcus terminalis which outlines the striatothalamic junction, where they may leave behind many embryonal remnants of interest in this study.

SOURCE OF EMBRYONAL RESTS IN THE STRIATOTHALAMIC REGION

During intrauterine development of the nucleus caudatus the matrix between this structure and the ventricular ependyma gradually reduces in volume; however, at birth there remains along the ventricular extent of the caudate nucleus a substantial subependymal layer of matrix. Within it there are cellular aggregations, which may be regarded as embryonal remnants. They are collections of spongioblasts and neuroblasts in various stages of maturation.

Their persistence in the region of the caudate nucleus coincides with the essentially divergent mode of development of the cortex pallii and

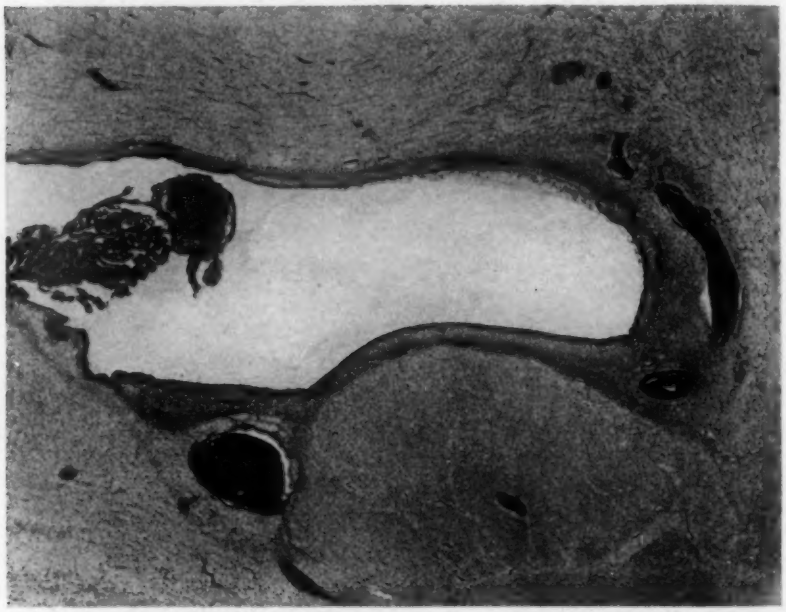

Fig. 4.—A coronal section of the brain of a newborn infant, showing the rostral end of the sulcus terminalis and the underlying subependymal cell plate, near the vena terminalis (× 5). The lamina affixa is also shown (left) extending from the choroid plexus to the floor of the sulcus terminalis.

that of the basal structures. During the formation of the cortex pallii, elements of the periventricular matrix migrate toward the surface to become arranged as the superficial gray matter, separated from the ventricle by the centrum semiovale. Cellular residues of the periventricular matrix may be observed in varying degrees in the subependymal zone of the human brain for some time throughout infancy, as shown by Schwarz, Goolker and Globus.[11] As this process of cell

11. Schwarz, H.; Goolker, P., and Globus, J. H.: Am. J. Dis. Child. **43**:889, 1932.

migration advances, the matrix gradually exhausts itself so that in time it disappears almost completely. Some matrix cells, however, are still found in the adult subependymal cell plate, as was pointed out by Opalski [11a] in his study on the wall of the adult ventricular system. With some of their subdivisions the basal ganglions remain in a permanent periventricular location, thus retaining their primitive ontogenetic

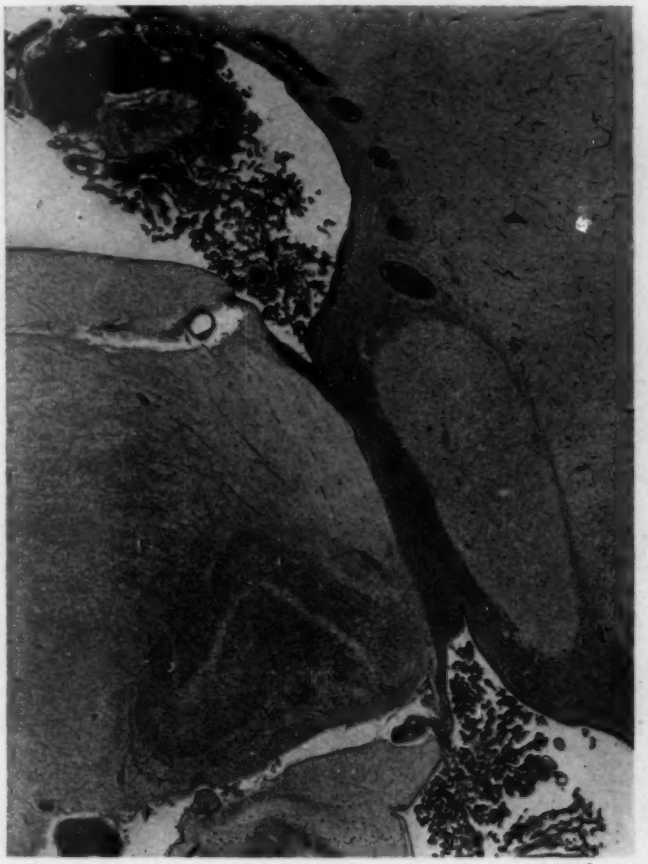

Fig. 5.—A coronal section of the brain of a newborn infant, showing the subependymal cell plate in the caudal bend of the sulcus terminalis (×7).

and topographic relations. The subependymal layer extends caudad along the sulcus terminalis between the thalamus and the caudate nucleus and can be followed into the descending horn of the lateral ventricle as far as the amygdaloid nucleus. Embryonal remnants are recognized particularly in the conspicuous thickening of the subependymal layer in the region of the interventricular foramen, at the rostral end of the

11a. Opalski, A.: Ztschr. f. d. ges. Neurol. u. Psychiat. **149**:221. 1934.

sulcus terminalis (fig. 4), while another thickening may be found in the caudal bend of this sulcus at the level of the nuclei of the pulvinar and the lateral geniculate body (fig. 5).

Another source of embryonal rests is provided by the process of partial coalescence in the anterior horn of the lateral ventricle. During the last months of intrauterine life, the extension of the lateral ventricle

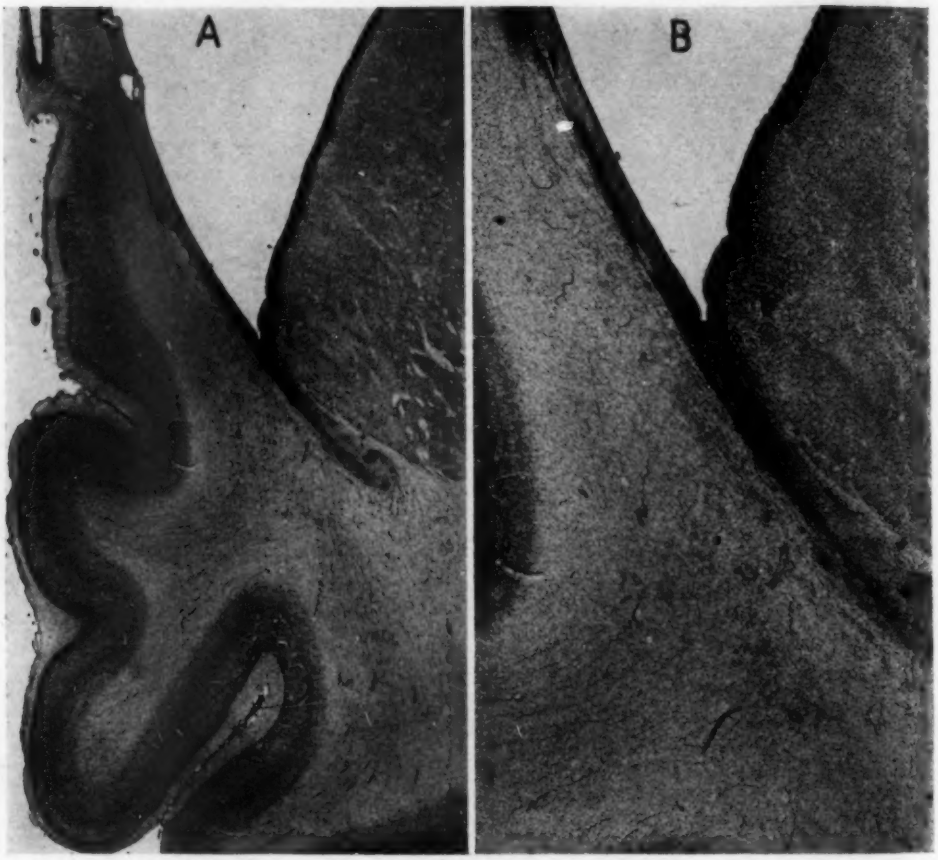

Fig. 6.—Coronal sections of the brain of a newborn infant, showing the obliterating process in the basal ventricular angle of the anterior horn of the lateral ventricle (*A*, × 5; *B*, × 10). The septum pellucidum is also shown with its subependymal plate.

into the olfactory stalk is obliterated by the fusion of its ependyma-lined walls. The adjacent ventral parts of the walls of the anterior horns also tend to fuse and thus obliterate the basal ventricular angle (fig. 6). Scattered groups of spongioblasts and ependymal cells are isolated by

this process below the ventricular surface (fig. 7). A layer of such embryonal remnants indicates the former ventricular extension into the rhinencephalon and spreads out below the head of the caudate nucleus.

Embryonal rests in the form of a distinct zone of subependyma are also found at the dorsal extremity of the ganglionic ridge around the dorsolateral angle of the lateral ventricle, between the caudate nucleus and the corpus callosum, bounded laterally by the fiber masses of the fasciculus subcallosus or fronto-occipitalis superior of Forel (fig. 4).

A thin subependymal layer likely to contain embryonal rests extends also along the ventricular wall of the septum pellucidum (fig. 6).

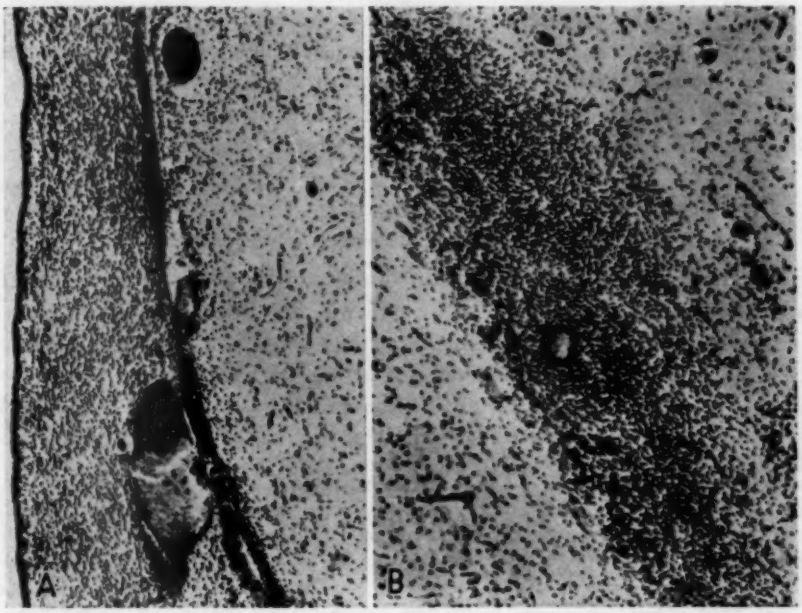

Fig. 7.—*A*, section of the brain of an infant 5 days old, showing the subependymal plate lining the third ventricle (×74.5.) *B*, section of the same brain, showing an embryonal rest detached from the subependymal plate (×88.5).

These four regions must, therefore, be considered as important sites of embryonal residues consisting of poorly differentiated cells of neuroectodermal derivation. They are to be added to other foci of embryonal remnants mentioned in the literature, such as the closing lines of the neural tube at the posterior median sulcus of the spinal cord, the aqueduct of Sylvius and its gray, the roof of the fourth ventricle, the periependymal plate of the fourth ventricle, the neurohypophysial stalk (Globus[12]) and the surface of the infundibulum bearing cell nests

12. Globus, J. H.: J. Neuropath. & Exper. Neurol. **1**:59, 1942.

related to the craniopharyngeal duct; the latter, of course, are not of neuroectodermal origin.

At this point, attention may be drawn to an observation by Allen [13] on cessation of mitosis in the central nervous system of the rat. He noted that in the brain of a rat a zone which he interpreted as a remnant of the mantle layer persisted along the lateral and dorsal walls of the lateral ventricles. The presence of this layer was observed by him in animals as old as 2 years. Essentially, it corresponds to our subependymal cell plate about the caudate nucleus. In describing this layer, Allen emphasized the fact that within it mitotic processes continued until a late period in the course of the development. Mitosis to a considerable degree of activity was noted by him as late as the twentieth postnatal day of the animal and to lesser degree as late as the one hundred and twentieth postnatal day.

The protracted mitotic activity is not out of accord with our own observations on embryonal rests in the human brain under discussion.

THE HISTOLOGIC CHARACTER AND THE INVASIVE FEATURES OF TUMORS IN THE STRIATOTHALAMIC REGION

Histologically, the 21 neoplasms of the striatothalamic region studied in this investigation fall into the following five interrelated categories: (*a*) spongioblastoma ependymale (3), (*b*) spongioblastoma multiforme (3), (*c*) spongioneuroblastoma (10), (*d*) transitional glioma (2), and (*e*) glioneuroma (3). All these are but variants of the larger so-called gliogenous group, or still better, members of the neuroectodermal group of brain tumors.

The study of the cellular elements and of their arrangement in the neoplasms was carried out on frozen, paraffin and celloidin (concentrated pyroxylin) sections, treated by several methods, such as hematoxylin-eosin, the Nissl stain, the Bielschowsky silver impregnation for neurofibrillae, the Globus modification of the Cajal gold chloride–mercury bichloride impregnation and the Hortega silver carbonate method. Hólzer's glia stain was also used in some cases, and in a number of instances Herxheimer's fat stain was employed. Of these methods, the Nissl and Bielschowsky methods proved to be especially effective for the detection of neuronal elements.

The invasive features of the tumors under investigation were determined by study of gross specimens aided by examination of serially prepared large celloidin sections which included the tumor and its surrounding structures. These sections were stained by a modification of Weigert's method for medullated fibers as well as by the Nissl, hematoxylin-eosin and Van Gieson methods. Sections were studied under low magnification, which permitted a better correlation of the gross with the microscopic features.

The cases selected for this investigation, it is hoped, will demonstrate the intimate relationship between the tumor formations and the

13. Allen, E.: J. Comp. Neurol. **22**:547, 1912.

embryonal remnants in the already enumerated locations. They will reveal also why those of the spongioneuroblastic and other spongioblastic varieties predominate in the series to be reported.

REPORT OF CASES

CASE 1 (Spongioblastoma ependymale).—A man aged 46 suddenly became unsteady of gait, falling to the left. A week later he experienced continuous buzzing in the right ear with complete deafness on that side. There followed decrease of memory, blurring of vision, and numbness and weakness in the right side of the face. He entered the hospital six weeks after the onset of symptoms.

On admission he showed a staggering gait, a positive Romberg sign with falling to the left, dysdiadokokinesis on the left, peripheral paresis of the right side of the face, deafness in the right ear, nystagmus in all directions, impairment of taste on the anterior two thirds of the tongue and absence of the pharyngeal reflex on the right side. The diagnosis was: a lesion on the right side of the pons and medulla involving the fifth, sixth, seventh, eighth and ninth cranial nerves.

Radiotherapy was administered, with resultant definite improvement; the patient was discharged about six weeks after admission. Six months later, severe frontal headaches returned, and weakness with stiffness of the right arm and leg developed. Impaired concentration and emotional instability brought him back to the hospital.

On the second admission, in addition to the previous findings there were hyperactive deep reflexes on both sides, bilateral dysdiadokokinesis, slow, slurred speech and coarse tremor and rigidity of the right arm, hand and foot, increasing under emotional stress.

Terminally, the severity of the symptoms increased, with advancing bilateral rigidity of the extremities and the development of a bilateral Babinski sign, accompanied by grasping reflexes. Somnolence passed into coma, terminating in death at the end of six weeks in the hospital.

Microscopic Anatomy.—The several foci of neoplastic growth were essentially similar in structure: They were densely cellular and only moderately vascularized. The predominant cell type was a primitive spongioblastic element, short carrot-like, cuboidal or low columnar in shape. Many of these cells were arranged in regular rows of an epithelial alinement, especially along and around blood vessels (fig. 8*A*). In some regions, neighboring on the lateral ventricle, the ependymal lining and the epithelium of the choroid plexus were directly continuous with these columns of epitheloid tumor cells. Rounded cells with isolated neuroblastic elements were found surrounded by less differentiated cells (fig. 8*B*). Multinucleated giant cells were present, but few. Mitotic figures were numerous. A reticular argentophil mesodermal stroma extended from the vascular walls into the tumor tissue. Small round cells with deeply staining nuclei were filling the adventitial spaces of many vessels and infiltrating neighboring tumor tissue. The borders of the neoplastic tissue displayed the invasive character of the growth. The floor of the fourth ventricle showed ependymal granulations, in which were thickenings of the subependymal cell plate containing cellular elements resembling those of the more frank neoplastic foci. Such ependymal granulations were also found in the lateral ventricle of the brain in case 8.

Gross Anatomy.—The right cerebellar hemisphere was enlarged, with its convolutions flattened. It contained a poorly defined large firm tumor mass. Microtopographic sections showed that the neoplasm was connected with the subependymal

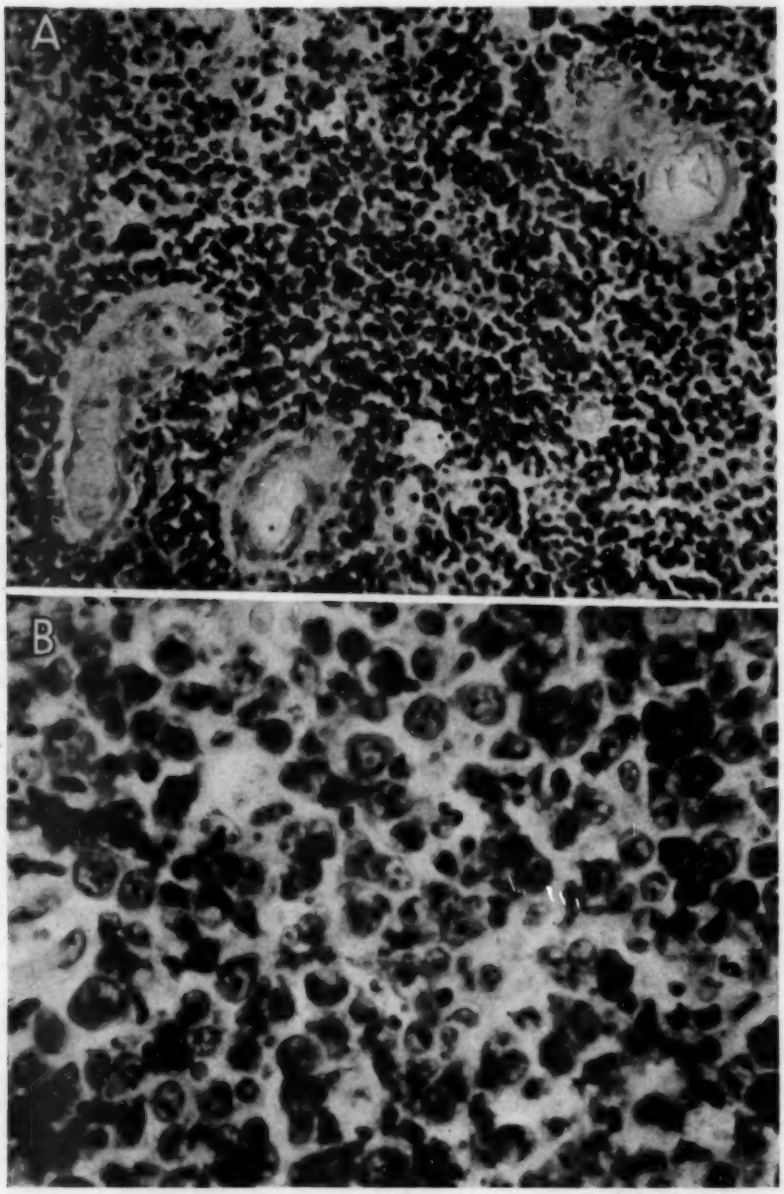

Fig. 8.—*A*, histologic character of the tumor in case 1, (spongioblastoma ependymale; ×200; hematoxylin-eosin). *B*, higher magnification of the tumor structure (×600).

cell plate of the right lateral recess of the fourth ventricle. There it completely destroyed the dorsal and ventral cochlear nuclei and invaded the homolateral vestibular nuclei. It spread to the medullary center of the right cerebellar hemisphere, involving the dentate nucleus. The tumor projected into the right pontofacial angle. The aqueduct was displaced dorsally, and the tegmentum showed linear hemorrhages in the midline. Ependymal granulations were seen in the region of the facial colliculus.

Fig. 9.—Coronal section of the tumor in case 1, showing neoplastic invasion of the corpus callosum and the underlying septum pellucidum (× 5).

In the cerebral hemispheres, a large tumor mass, implicating the posterior part of the septum pellucidum (fig. 9) near the interventricular foramen, had invaded the corpus callosum and the fornix. This mass extended from about 0.5 cm. rostrad to the anterior commissure, backward to within 1 cm. of the splenium. It was connected with the subependymal cell plate of the septum pellucidum and the left choroid plexus of the lateral ventricle (fig. 10). This main tumor had a rostrocaudal length of about 3.5 cm. and a transverse diameter of about 4 cm. In addition to this there was a neoplastic focus, about 0.5 cm. in diameter and more

than 2 cm. in length, involving the right caudate nucleus in the region of the interventricular foramen. Another neoplastic focus of similar size was found along the rostral end of the left sulcus terminalis, extending medially into the surface of the lateral and the medial nucleus of the thalamus. Each taenia thalami showed at its rostral end a minute neoplastic focus, best recognized under the microscope. It was connected with the subependymal cell plate of the third ventricle.

In a survey of case 1, there are two significant observations to be recorded. One has reference to the histologic character of the tumor. The component cell forms indicate a low stage of differentiation. However, the cell arrangement suggests an ependymal orientation. Moreover, this interpretation is supported by the intimate connection of the

Fig. 10.—Camera lucida drawing showing the distribution of the tumor in case 1.

tumor with the ependyma and the choroid plexus. The tumor is therefore grouped with the so-called spongioblastoma ependymale in spite of the presence of neuroblastic elements among other cell forms.

The topographic features are also important, for they reveal close relations to the subependymal cell plate in the several zones in which embryonal residues are common, in several widely separated parts of the brain.

The neoplastic focus in the lateral recess of the fourth ventricle bears a strong resemblance to a tumor in the cerebellopontile angle described by Alexander [14] and designated by him as medulloblastoma.

14. Alexander, L.: A. Research Nerv. & Ment. Dis., Proc. (1935) **16**:266, 1937.

The multiplicity of neoplastic processes in a single case is, of course, in harmony with the concept that multiplicity of germinal centers is responsible for the corresponding dissemination of the tumor.

CASE 2 (Spongioblastoma ependymale).—The patient was a woman aged 35. She was subject to epileptiform attacks of jacksonian character at the age of 20. The seizures were limited to the left side of the body and were not accompanied by loss of consciousness. They recurred every two months. In the last year before she entered the hospital, mental changes developed; she became irritable and incoherent and expressed ideas of persecution.

On admission she was confused, agitated and disoriented, and displayed marked impairment of memory. The pupils were unequal, the left larger than the right; they reacted well to light and in accommodation. There were bilateral papilledema, left spastic hemiparesis and deviation of the tongue to the left. There were also left hemihypalgesia and hypesthesia. All the deep reflexes were exaggerated, the left abdominal reflexes were absent and there was a bilateral Babinski sign.

With the diagnosis of a right hemispheric tumor, exploratory craniotomy was performed. A tumor of the right hemisphere was disclosed, but the surgeon also noted several hard nodules in the neighborhood of the tumor and suggested the diagnosis of tuberous sclerosis. The patient died the following day.

Microscopic Anatomy.—The tumor was fairly densely cellular and moderately vascularized with some areas of softening. The predominant cell type was a medium-sized to large, irregularly shaped spongioblast. There were, however, numerous cells varying in shape from cuboidal to low columnar, arranged in regular rows along and around vessels. Giant cells were scarce. Mitotic figures were also few. The periphery of the tumor showed an infiltrating type of growth. In the areas of the frontoparietal cortex monster cells characteristic of tuberous sclerosis were found.

Gross Anatomy.—The convolutions of the right hemisphere were markedly flattened. In the middle third of the middle and the superior frontal gyrus of the right hemisphere there was a softened area which represented the free surface of a large infiltrating tumor extending deep into the brain substance. It was surrounded by an area of increased resistance, especially in the proximity of the frontal pole. The tumor mass had invaded the medullary center of the right frontal lobe and compressed the anterior horn of the lateral ventricle on that side. It had completely replaced the septum pellucidum (fig. 11) and the right anterior pillar of the fornix, infiltrating into the adjacent parts of the corpus callosum and extending across the midline. The neoplastic growth involved the subependymal cell plate of the septum pellucidum, of the nucleus accumbens and also the region of late fetal coalescence basal to the anterior horn of the right lateral ventricle and basal to the head of the right caudate nucleus.

The cellular structure of the tumor in case 2 places it in the same group as the tumor in case 1. The tumor is therefore spongioblastoma ependymale. The cell form of the tumor is of a higher degree of differentiation. The cellular processes are more numerous, and the arrangement of the cells is less regular. Thus, it assumes an orientation toward spongioblastoma multiforme, but of great significance are the distribution of the tumor and the presence of the growth in the septum pellucidum, affecting both its sides and from there extending toward the zone of

coalescence of the anterior horn of the lateral ventricle. Thus, we find here an example in which the subependymal plate at a crucial point is the nucleus for tumor formation.

Of significance also is the fact that this patient was subject to epileptiform attacks at the age of 20, which in the light of subsequent findings may be assumed to indicate that some blastomatous malformation was

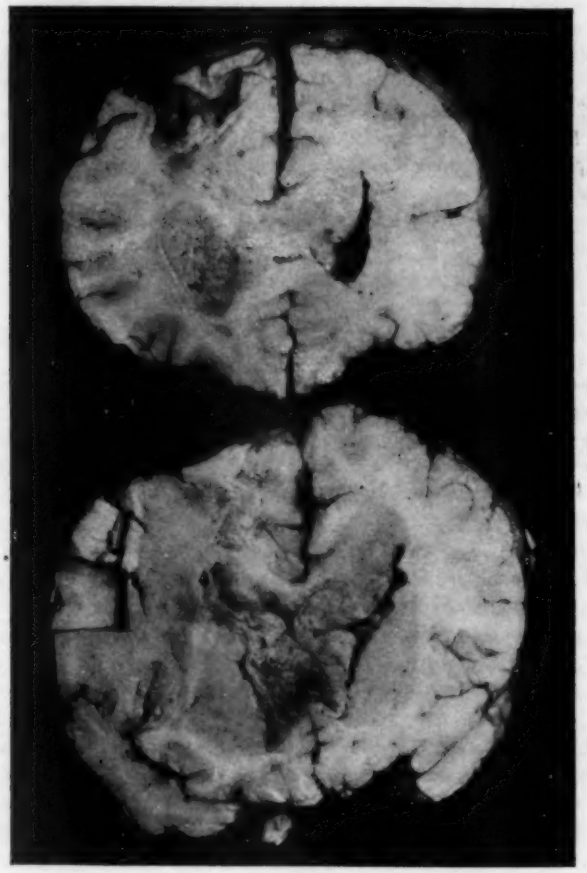

Fig. 11.—Coronal section of the brain in case 2 (spongioblastoma ependymale), showing the location and the appearance of the tumor.

already then in existence. The presence of monster cells in the cortex in an area remote from the site of the tumor is also an important finding and is in keeping with both the clinical manifestations of a convulsive state and the anatomic evidence of a disseminated primitive neuroectodermal tumor.

CASE 3 (Spongioblastoma ependymale).—A woman aged 39 had experienced headaches increasing in intensity for one year; shortly before admission to the hospital there were stiffness of the neck and pain on moving it, insomnia, repeated vomiting and impairment of vision.

On admission the findings were: bilateral papilledema with retinal hemorrhages; deviation of the tongue to the right; atrophy and fibrillations of the right side of the tongue; grimacing and choreiform movements of the extremities; suggestion of right hemiparesis; depressed deep reflexes; emotional instability. The diagnosis was: a cerebral neoplasm in the neighborhood of the third ventricle invading the basal ganglions.

Terminally there was a sudden decline with a rise in temperature, followed by coma ending in death one week after admission. There was no surgical intervention.

Microscopic Anatomy.—The tumor was densely cellular and richly vascularized, with large areas of degeneration and extravasation. The cells were mainly spongioblasts of various shapes with a large quota of characteristic multinucleated giant cells (fig. 12*A*). Near the areas of necrosis many of the spongioblasts showed swollen hyalin-like cytoplasm, not unlike that of Nissl's fattened glia.

In the proximity of the sulcus terminalis there was a fairly wide zone of cellular aggregation which bore a striking similarity in its arrangement to the cell organization in selected areas of the subependymal zone of late embryonal brains and to the cell forms and the cell arrangement in ependymoma, particularly of the less differentiated type. It was characterized by circular aggregations of many-layered cells, low columnar in type (fig. 12*B*). Within the center of each aggregation there was usually found a group of vessels. The structure was not unlike that of a budding choroid villus and one that is often described as a pseudorosette. Some suggested that the vessels had reached subependymal cysts secondarily, simulating structures of glomerular appearance.

Gross Anatomy.—The tumor was a large oval mass, measuring about 4.5 cm. in its longest diameter. It was connected with the posterior part of the left sulcus terminalis where the latter at the level of the pulvinar bends ventrad (fig. 13). The neoplasm, distinctly infiltrating in character, invaded and almost completely replaced the left pulvinar. Rostrad it extended along the sulcus terminalis into the dorsolateral and medial thalamic nuclei toward the midline. Large parts of the ventral thalamic nuclei were left intact but were compressed and displaced in a ventrolateral direction. The left internal capsule and adjacent parts of the lenticular nucleus were similarly compressed and displaced. The tumor invaded also the left fornix, posterior parts of the corpus callosum and the pineal body. The subependymal cell plate of the third ventricle where it was approached by the tumor was represented by a thickened and spongy edematous band. Cell proliferations from this zone bridged the ventricle in some places to join the opposite (right) subependymal cell plate, which showed moderate thinning in its dorsal portion. The third ventricle in its uninterrupted portions was reduced to a narrow slit. Both lateral ventricles were moderately distended in all subdivisions, slightly more on the left than on the right side; the posterior part of the body of the left ventricle, however, was reduced in size by the direct pressure of the tumor.

Of significance in case 3 is the histologic pattern of the tumor. It bears a stronger similarity to the ependyma and its derivatives (choroid plexus) than that found in case 1. When this observation is coupled with the recognition of the tumor's intimate relation to the subependymal

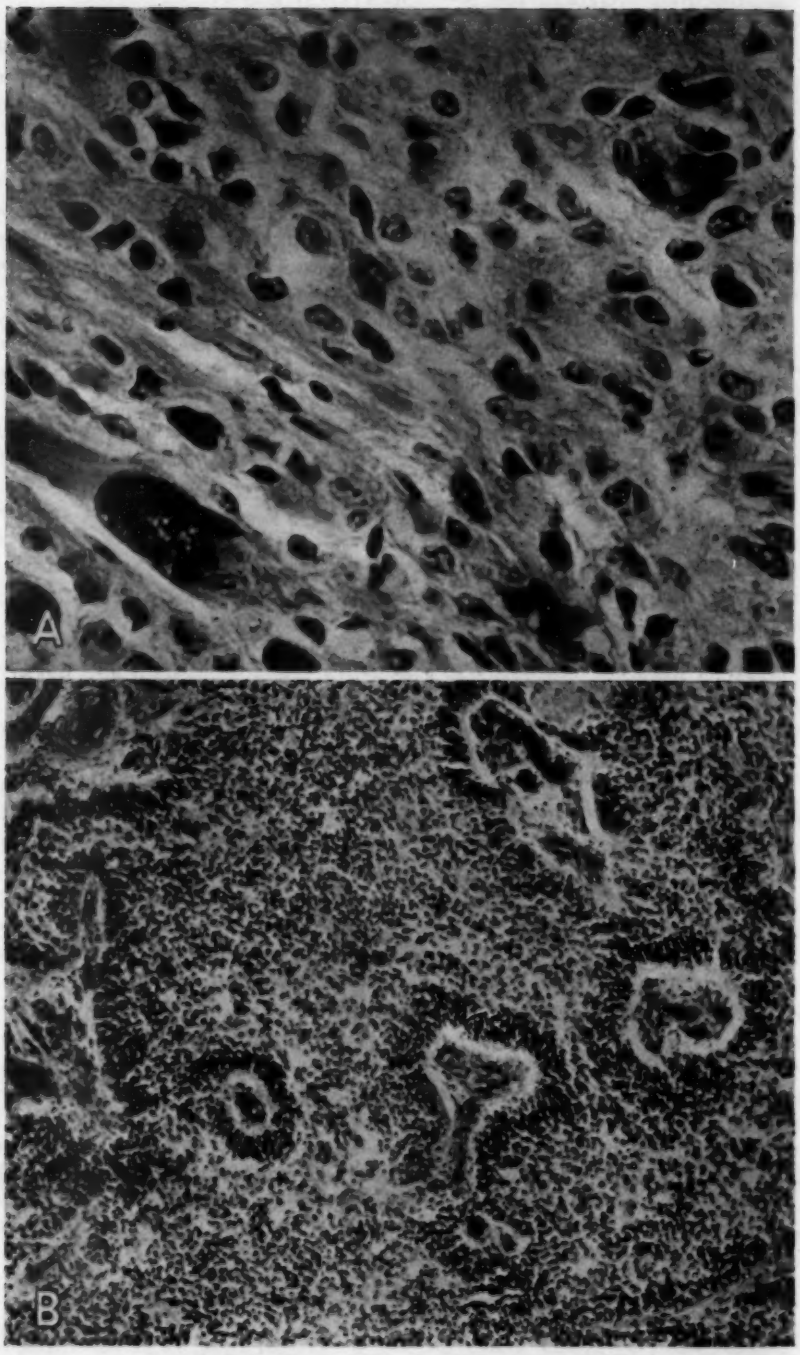

Fig. 12.—Histologic character of the tumor (spongioblastoma ependymale) in case 3. In *A* several typical giant cells are seen (× 545). In *B* the characteristic cell arrangement, with aggregation of the cells about blood vessels, is shown (× 90).

plate along the course of the sulcus terminalis, the deduction is obvious that we are dealing with a tumor which most probably had its origin in embryonal residues commonly encountered in this location. While this neoplasm is closely related to and almost merges with spongioblastoma

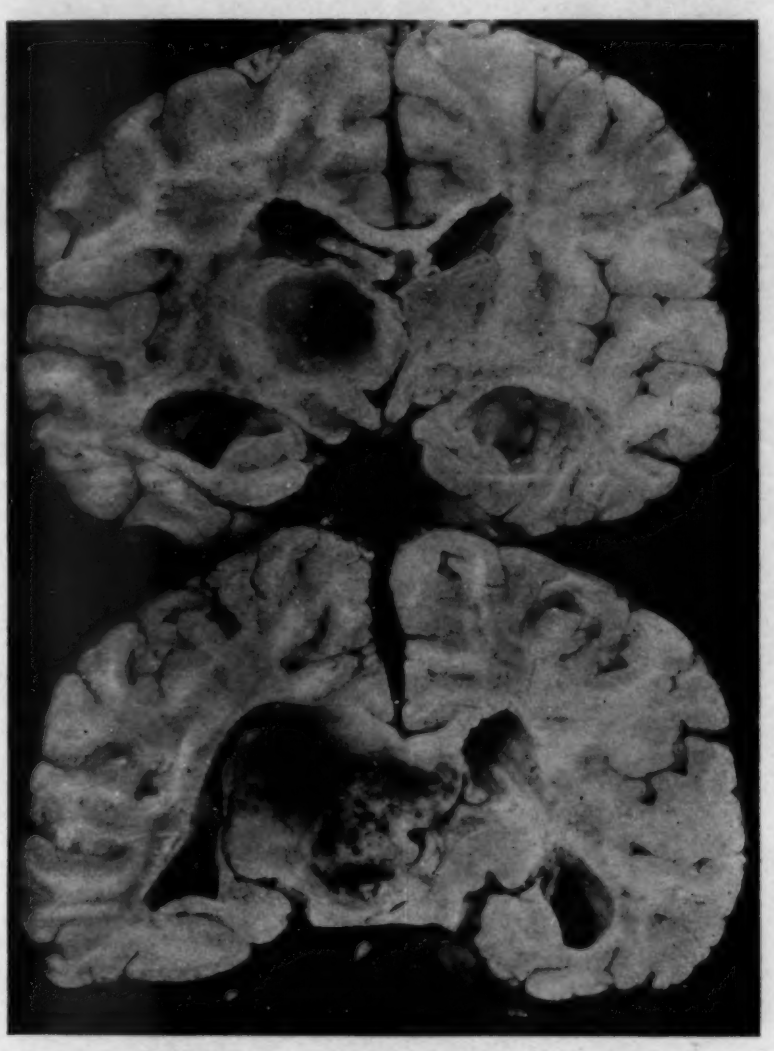

Fig. 13.—Coronal section of the brain, showing the gross appearance of the tumor (spongioblastoma ependymale) in case 3 and its intimate relationship with the posterior bend of the sulcus terminalis (compare with fig. 5).

multiforme, it nevertheless is nearer to the ependymomatous variety and could be named spongioblastic ependymoma or spongioblastoma ependymale.

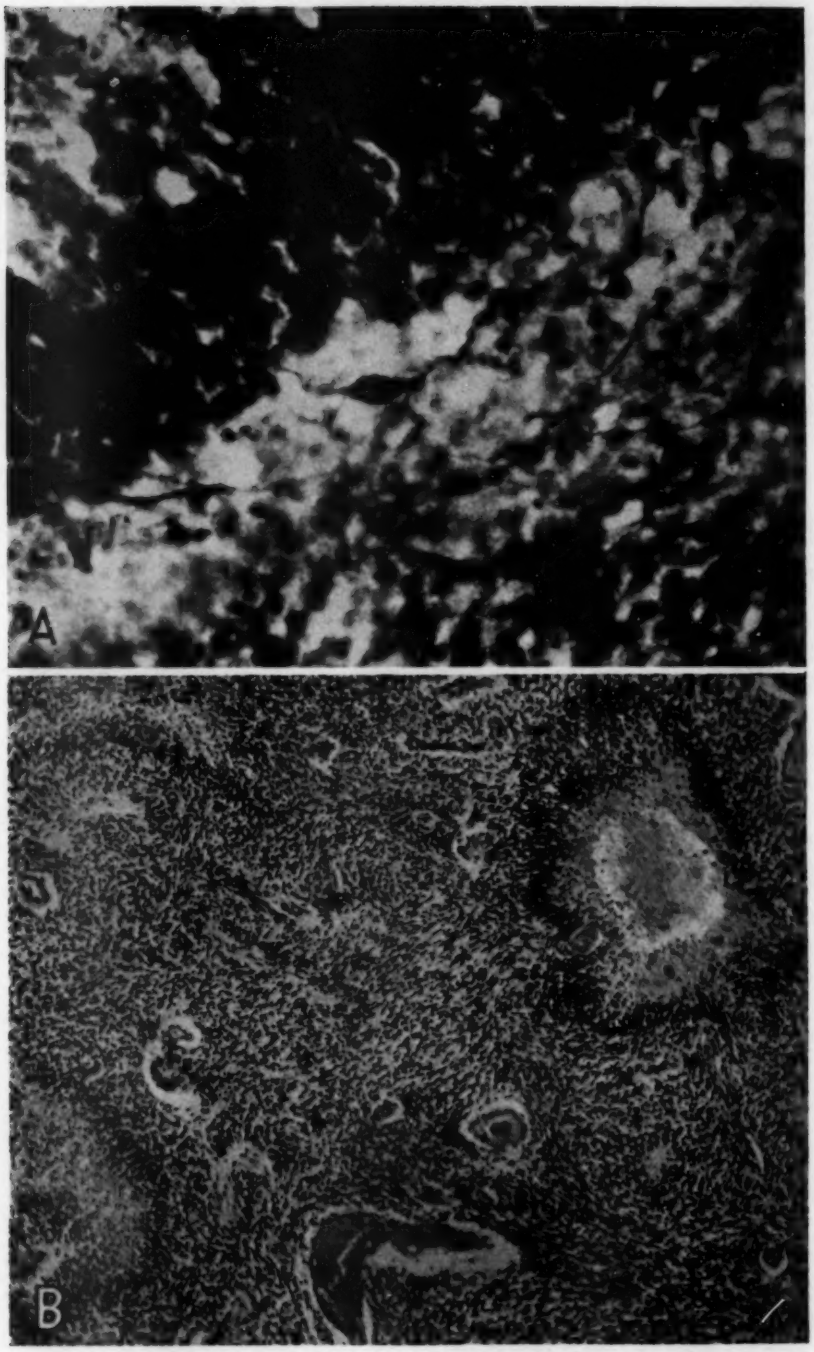

Fig. 14.—*A*, histologic character of the tumor in case 4 (spongioblastoma multiforme), showing bipolar spongioblasts (Bielschowsky silver impregnation; ×350). *B*, histologic character of the tumor in case 5 (spongioblastoma multiforme), showing the palisading of nuclei about the central areas of necrosis (×185).

CASE 4 (Spongioblastoma multiforme).—A woman aged 48 years complained of impairment of vision, more marked on the right, of three weeks' duration, accompanied by generalized weakness and impairment of memory.

On admission the findings were: drowsiness; motor aphasia; impaired vision, greater in the right eye; normal fundi; unequal pupils, the right greater than the left, both irregular, both fixed to light and in accommodation; the right eye in slightly divergent and downward position; paralysis of the upward gaze; slight supranuclear weakness of the right side of the face; generalized hyperreflexia, greater on the right than on the left; a Babinski sign on the right; slight right-sided hypalgesia and hypesthesia. Studies of the cerebrospinal fluid gave negative results. The diagnosis was: a lesion near the pineal body involving chiefly the left side of the brain stem. Ventriculography revealed dilated lateral and third ventricles and block to passage of air to the iter. The patient showed a gradual decline terminating in death five days after admission. There was no surgical intervention.

Microscopic Anatomy.—The tumor was of substantial cell density and contained an unusual number of capillaries. Its predominant cells, the spongioblasts, were arranged in palisades and clusters around necrotic areas. Silver stain showed many typically bipolar spongioblasts (fig. 14*A*). Multinuclear giant cells and mitoses were frequent. Two features in the tumor were quite striking: one was an extensive network of reticulum fibers forming a delicate stroma between the tumor cells, particularly in the vicinity of blood vessels, where this reticular network was found to be directly continuous with reticular fibers in the adventitia, while the other was the marked hypertrophy and proliferation of the adventitial cells, which in places formed thick, partly syncytial cell bands around the smaller vessels. The latter often fused with the adventitial cells. The lumens of such vessels were often greatly narrowed. Numerous multinuclear giant cells and many mitotic figures were seen.

Gross Anatomy.—A tumor was found occupying the side of the tectum and tegmentum of the midbrain, extending as far as the left peduncle anteriorly, the hypothalamic region ventrally and the pulvinar dorsally, reaching the posterior part of the sulcus terminalis. The anterior portion of the tumor assumed an irregularly round shape, about 1.5 to 2 cm. in diameter. The posterior part of the tumor tapered out toward the midpontile region. There was marked bilateral symmetric hydrocephalus.

This tumor is more typical of spongioblastoma multiforme; on the other hand, it is particularly rich in fibers of mesodermal derivation. Its location is less conspicuously related to the sulcus terminalis but is still within the limits of the regions containing embryonal residues.

CASE 5 (Spongioblastoma multiforme).—A man aged 59 noted impairment of memory four weeks before admission, followed by increasing difficulty in finding the right words for expression; three weeks later there was reduction of power in the right hand and foot.

On admission the findings were: depressed mood; motor aphasia and anomia with perseveration; right hemiparesis; dysdiadokokinesis on the right; slight hyperreflexia in the right upper extremity; a Babinski sign on the right; absence of abdominal reflexes on the right; cerebrospinal fluid under initial pressure of 180 mm. of water, xanthochromic fluid. The diagnosis was: a tumor in the left cerebral hemisphere.

The terminal events were: advancing apathy and aphasia; a sudden decline; after an enema, deep stupor; death five days after admission, without surgical intervention.

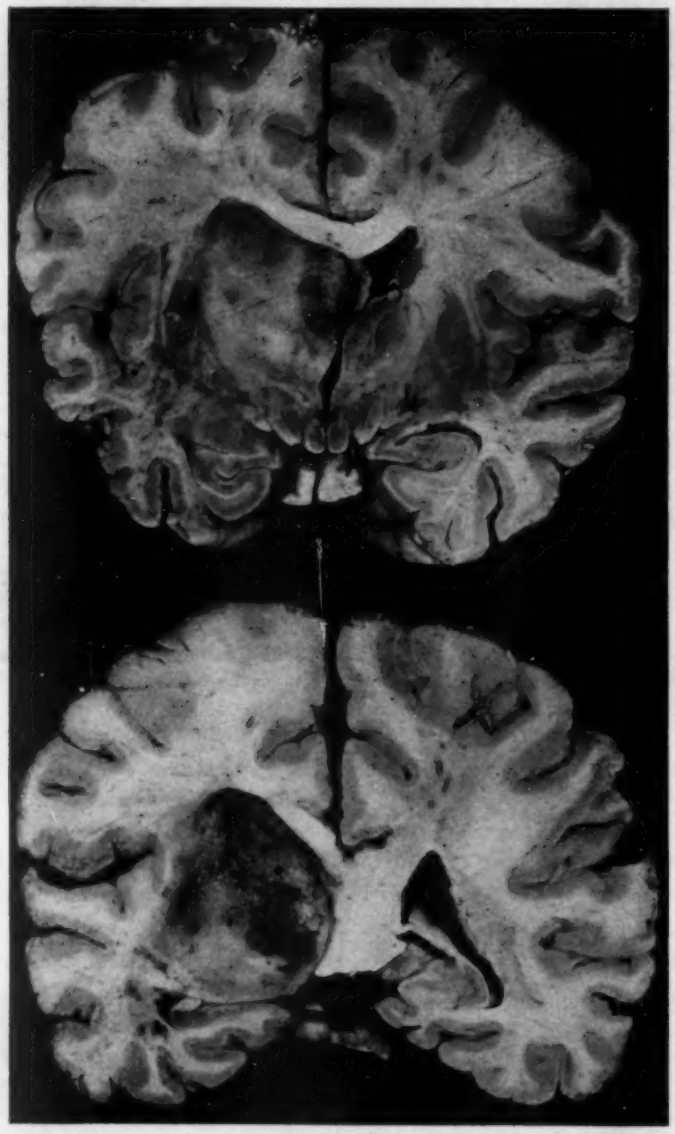

Fig. 15.—Gross appearance of tumor in case 5 (spongioblastoma multiforme). Note the obliteration of the dorsal portion of the left sulcus terminalis by the invading tumor.

Microscopic Anatomy.—The tumor was densely cellular and very vascular. Spindle-shaped, elongated bipolar spongioblasts were dominant. The tumor was

essentially one of the spongioblastoma multiforme variety, although it was devoid of the more characteristic multinucleated giant cells. However, at various places small spongioblasts could be seen grouped so that their several nuclei lay close together. These structures may possibly have represented stages of multinuclear giant cells and could be interpreted as evidence of giant cells undergoing plural amitosis or pluripolar mitosis. The tumor cells formed palisades (fig. 14 *B*) and irregular circles around necrotic areas. The hypertrophy of endothelial and adventitial cells was quite marked. There was also a conspicuous increase in reticulum fibers in the vascular walls, but in contradistinction to the findings in case 2 these fibers did not invade the tumor tissue proper.

Of interest was the presence of a large group of nerve cells which had retained their normal distribution and normal individual character in an area best described as that of the nucleus ventrolateralis of the thalamus. This is pointed out here so that it will be recognized that non-neoplastic nerve cells may be found retaining a normal position, number and configuration in the midst of an infiltrating primary neuroectodermal tumor.

Gross Anatomy.—The tumor was connected with the entire dorsal portion of the left sulcus terminalis (fig. 15). Rostrally it had invaded the anterior part of the left thalamus, including the anterior nucleus within the tuberculum anterius. In this region, however, the internal capsule and the lenticular nucleus remained intact. Caudad the neoplasm infiltrated the entire thalamus as well as the internal capsule and the lenticular nucleus, extending into the medullary center of the insula, the anterior part of the temporal lobe and the anterior third of the occipital lobe. The optic chiasma was deviated to the right.

In this additional instance of spongioblastoma multiforme, the sulcus terminalis was obliterated by virtue of the active and massive participation of the embryonal residues within its domain.

Case 6 (Spongioblastoma multiforme).—A woman aged 44 had experienced difficulty in concentration, dizziness and somnolence of increasing severity for four weeks and shaking of the right arm and drooping of the right side of the face for four days.

On admission the findings were: semistupor; slight right hemiparesis; fundi within normal limits; a suggestion of right homonymous hemianopia; sluggish pupillary reaction to light; deviation of the left eye to the left; weakness of the right side of the face; right-sided hyperreflexia; a Babinski sign on the right; clonus of the right ankle and patella; hypalgesia on the right; cerebrospinal fluid under an initial pressure of 170 mm. of water, containing 140 lymphocytes. The diagnosis was: a tumor of the left frontal lobe.

Shortly after the lumbar puncture there developed coma, convulsive seizures and signs of bilateral involvement of the pyramidal tracts. A bilateral exploration was made by way of trephine openings; no tumor was disclosed. Death occurred one day after admission.

Microscopic Anatomy.—The tumor was very cellular and richly vascularized, with areas of degeneration and extravasation. The cellular elements were represented by irregularly shaped spongioblasts alternating with a large number of multinucleated giant cells (fig. 16 *A*). Numerous mitotic figures were seen, many of which were atypical. Some of the latter were in the nature of multipolar mitoses (fig. 16 *B*) within giant cells and served as definite evidence that such multinuclear elements develop through this form of mitosis. The blood vessels were thin walled and rather large.

Gross Anatomy.—The neoplasm (fig. 17) was a very large mass in the left cerebral hemisphere, joined to the sulcus terminalis at the level of the middle third of the caudate nucleus. From there it extended into the adjacent parts of the

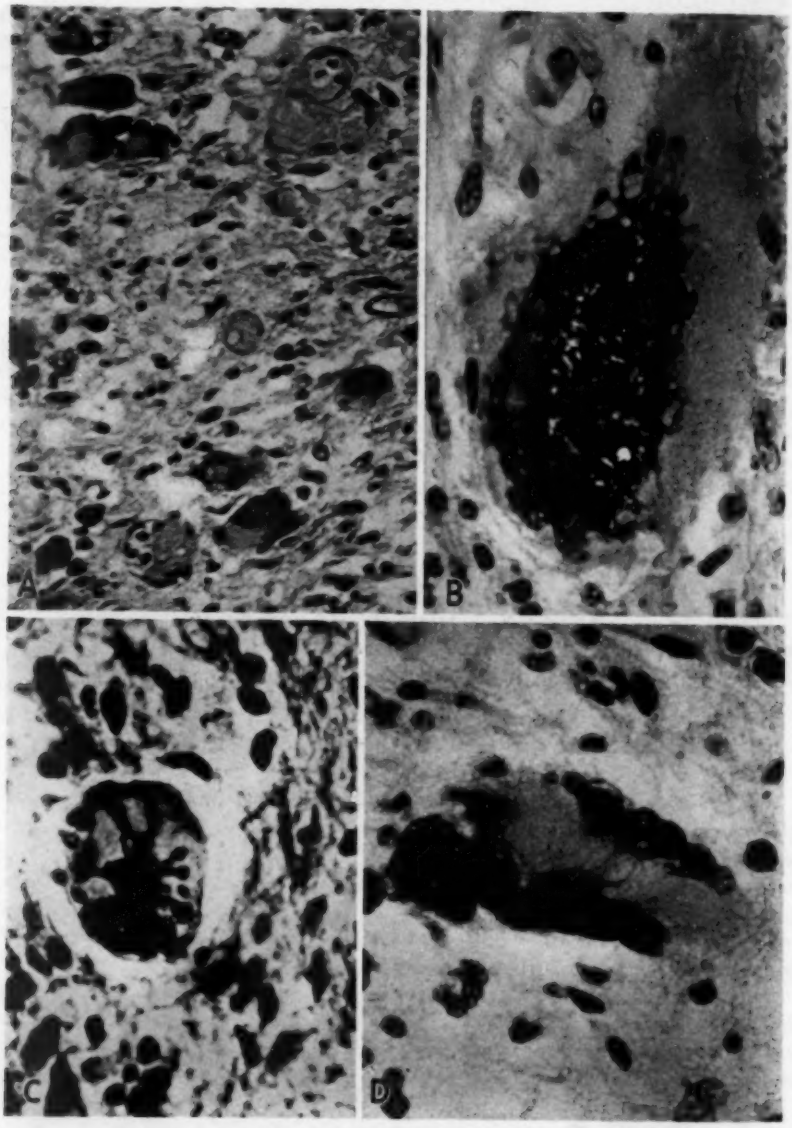

Fig. 16.—*A*, section of the tumor in case 4, showing numerous giant cells (×294.5). *B*, giant cell showing multipolar mitosis (×506). *C*, giant cell showing nuclear extensions (×631). *D*, giant cell showing nuclear threads (×506).

telencephalon as well as those of the diencephalon. In the former the internal capsule and the lenticular nucleus were partly compressed and partly invaded. Both walls of the septum pellucidum were almost completely replaced by tumor tissue. In the diencephalon the thalamus was invaded and almost completely replaced by the tumor, which extended beyond this structure into the rostral part of the mesencephalic tectum.

Of significance in case 6 is the substantial participation of the septum pellucidum and the structures adjacent to the sulcus terminalis in the formation of the tumor.

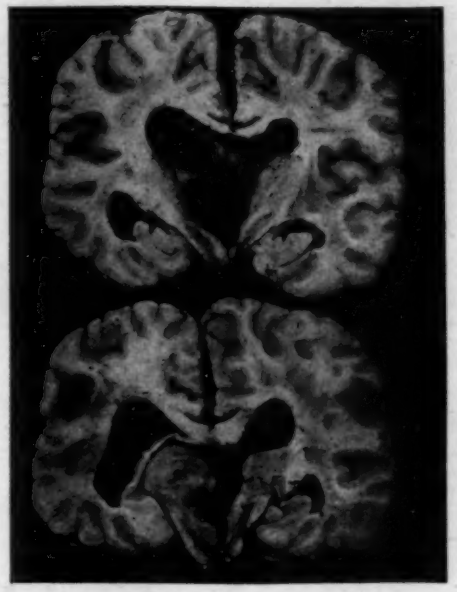

Fig. 17.—Coronal sections of the brain showing the gross appearance and the location of the tumor in case 6 (spongioblastoma multiforme).

CASE 7 (Spongioneuroblastoma).—A woman aged 48 alleged that she had suffered an injury to the head six months before admission, when she struck her head on the door of a car. Two weeks before admission severe pain developed in the right side of the face, including the right upper jaw. A molar tooth was extracted without relief. A week before admission the pain in the face disappeared simultaneously with onset of severe persistent headache, dizziness accompanied by paralysis of the right side of the face and weakness of the left leg.

On admission the findings were: drowsiness, with slow speech and mental retardation; normal fundi; suggestive blurring of the nasal margins of both disks; paresis of the right external rectus muscle; central facial palsy on the right; left spastic hemiparesis; increased knee and ankle jerks on the right; a positive Babinski sign on the right; diminished left knee and ankle jerks; absence of abdominal reflexes. The diagnosis was: a subdural hematoma or neoplasm in the right frontotemporal lobe. Bilateral occipital trephination revealed no lesion. A ven-

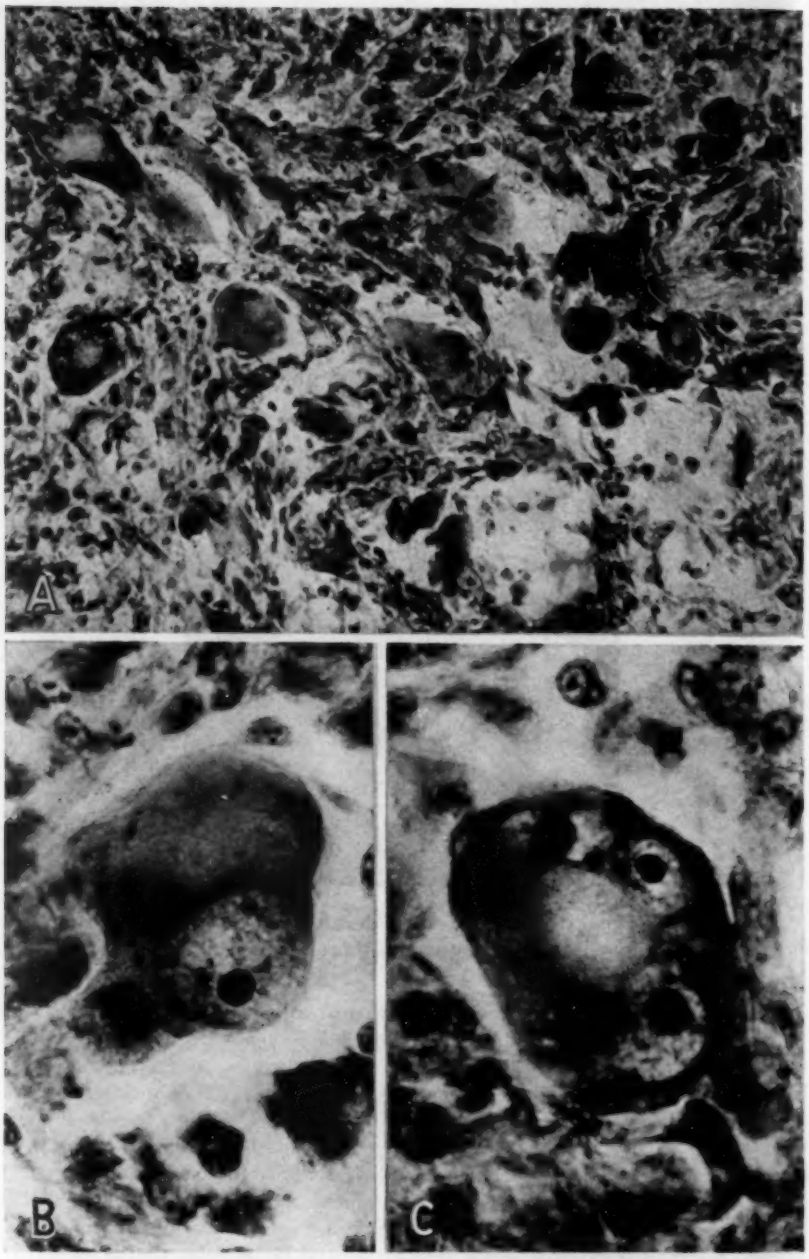

Fig. 18.—*A*, histologic appearance of tumor in case 7 (spongioneuroblastoma), showing numerous neuroblastic giant cells (Nissl preparation; ×189). *B*, giant neuroblast with a single nucleus (×656.4). *C*, giant multinuclear neuroblast (×661).

triculogram showed the interventricular septum displaced to the left, the left lateral ventricle dilated and the right compressed.

Exploratory craniotomy was performed in search of a cerebral neoplasm in the right frontal region. An aspiration needle entered a cyst, with fluid and fragments of brain tissue gushing forth. During the right subtemporal decompression, tumor tissue was found at a depth of 3.5 cm. in the frontotemporal region. The patient died one hour after operation.

Microscopic Anatomy.—The tumor was very cellular and richly vascular with numerous rather wide and thin-walled blood vessels. The cells were arranged in

Fig. 19.—Horizontal section of the brain in case 7 (spongioneuroblastoma), showing the distribution of the growth.

strands and islands, their nuclei varying widely in form, size and chromatin content. Multinuclear giant cells were frequent, and many mitotic figures were found (fig. 18 *A*). Bielschowsky preparations showed nests of irregularly shaped neuroblasts, many of them multinuclear; immature nerve cells were also revealed by the Nissl stain. Bipolar spindle-shaped spongioblasts were very numerous. The vascular pattern consisted of looping and richly ramifying blood vessels of the sinusoid type, with walls of various thickness, often with endothelial and perithelial cells growing out bandlike into the tumor tissue. Considerable necrosis was found in parts of the neoplasm.

Gross Anatomy.—A large area of discoloration, indicating the site of the tumor, was found occupying the right cerebral hemisphere (fig. 19). The anterior boundary of the neoplasm corresponded to a plane about 2 cm. anterior to the

corpus callosum. Laterally the tumor occupied almost the whole width of the hemisphere, leaving only a narrow peripheral margin of cerebral tissue. Ventrally it had reached the anterior pole of the temporal lobe, the lenticular nucleus and the island of Reil. Anteriorly it was connected with the head of the caudate nucleus in the region basal to the anterior horn of the lateral ventricle at the site

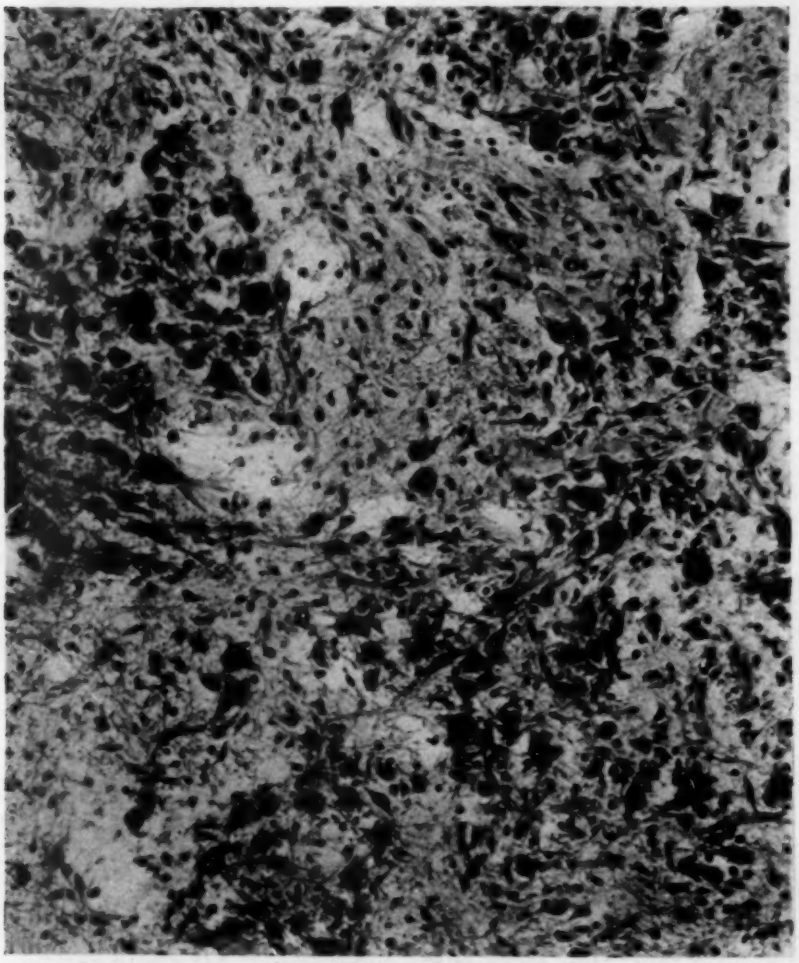

Fig. 20.—Histologic character of the tumor in case 8 (spongioneuroblastoma). Note the basket-like arrangement of the tumor cells (× 120).

of the late fetal coalescence of the ventricular extension into the rhinencephalon. Caudally it involved an extensive portion of the sulcus terminalis.

This is a striking example of apparently multiple tumor formation which on further investigation is found to be a single mass extending from the region of the anterior horn coalescence to and through the sulcus terminalis. The tumor is an outspoken spongioneuroblastoma with typical giant neuroblasts.

CASE 8 (Spongioneuroblastoma disseminatum [tuberous sclerosis]).—The patient was a girl aged 16. In early life she had convulsions. For two years before admission to the hospital there were cramplike pains in the legs. Persistent frontal headaches and impairment of vision, increasing in intensity, were of seven months' duration; for two months there had been veering to the right in walking.

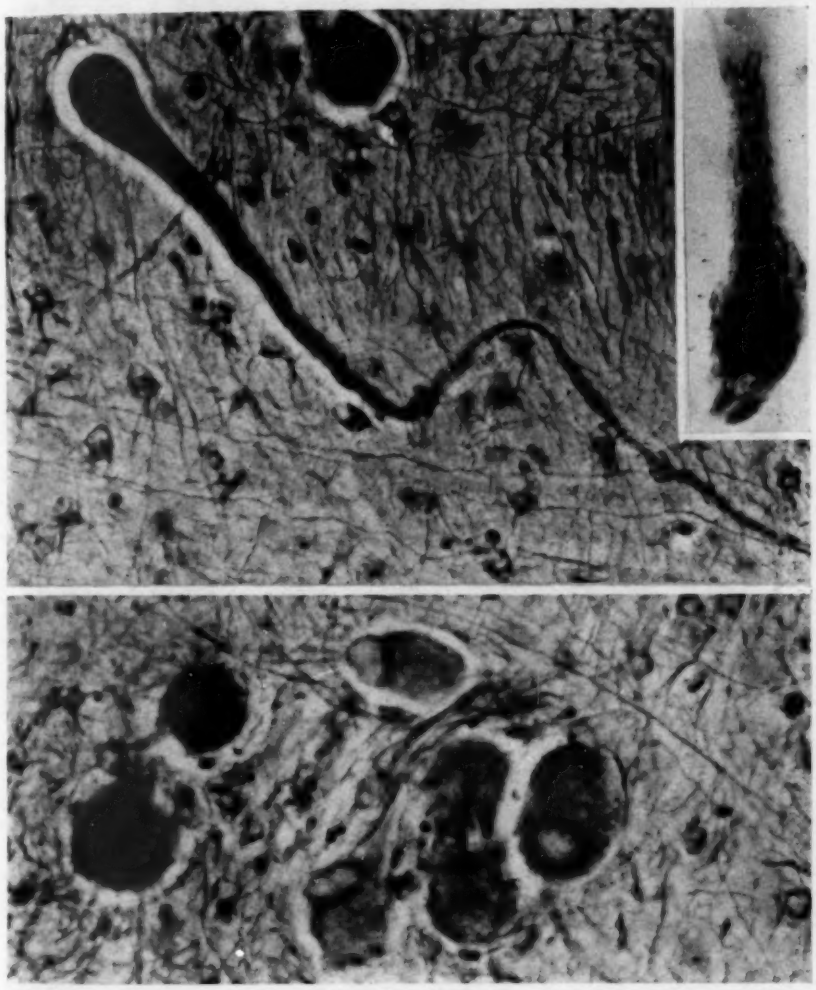

Fig. 21.—Types of monstrous giant cells in nodules of "tuberous sclerosis" in case 8 (spongioneuroblastoma) (× 201).

On admission she had a diffuse sebaceous eruption on the face, was retarded mentally and tilted the head to the left in walking. There were bilateral exophthalmos, bilateral papilledema, more marked in the left eye, and diminution of the corneal reflexes. There were central weakness of the left side of the face and hypotonia of both upper extremities. The diagnosis was: tuberous sclerosis with an expanding lesion in the posterior fossa.

There were no further changes for nine months; then during the next three months progressive dimness of vision, vertigo and periodic vomiting were noted. The pupils were dilated and reacted sluggishly to light. A ventriculographic examination was followed several hours later by coma; death occurred the next day.

Microscopic Anatomy.—The tumor displayed a nestlike arrangement of undifferentiated, irregularly formed cell elements of both the spongioblastic and the

Fig. 22.—Gross appearance of a horizontal section of the brain in case 8 (spongioneuroblastoma), showing the seat of the tumor and the ependymal granulations with one large subependymal granule in the posterior horn.

neuroblastic variety (fig. 20). Necrotic areas and concretions were abundant. Giant cells, some of which were clearly recognizable as nerve cells in Nissl preparations, were numerous. In some parts of the tumor many of these nestlike cell clusters were separated from one another by irregular strands of myelinated fibers and contained predominantly cells of neuroblastic lineage, while in other parts of the tumor spongioblastic elements prevailed. In many regions of the cerebral cortex there were areas of tuberous sclerosis (fig. 21).

Gross Anatomy.—The main tumor mass measured 2.5 cm. in its anteroposterior diameter and was situated in the most rostral portion of the right sulcus terminalis, where the latter ends at the interventricular foramen (fig. 22). The tumor mass, which extended as far as the massa intermedia, compressed the third ventricle from above downward. Two much smaller tumors were found in the left hemisphere in two separate regions of the left diencephalotelencephalic boundary zone, one in the middle part of the sulcus terminalis, the other in the caudal part of the same

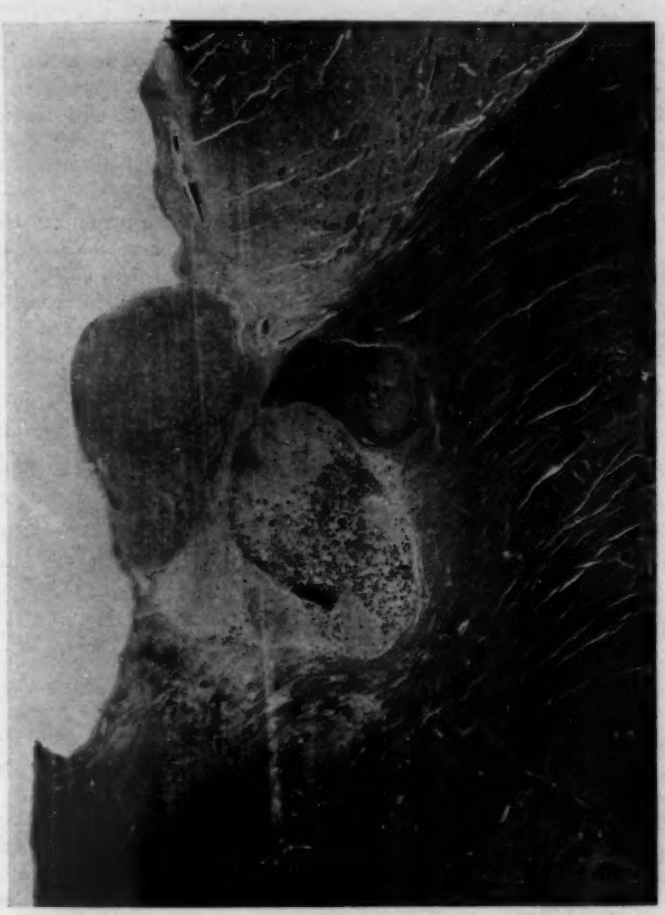

Fig. 23.—Section of the tumor mass in relation to the anterior end of the sulcus terminalis near the knee of the internal capsule in case 8 (spongioneuroblastoma). (The black granules are calcium deposits.) The nucleus caudatus is seen above, while the nucleus anterior and the nucleus lateralis are seen below (× 10).

sulcus close to the left pulvinar. The lateral ventricles were distended and filled with bloody fluid, while the dorsal part of the third ventricle was reduced to a narrow slit by the opposing tumor masses.

Although it appears from the gross description that the main tumor had replaced the anterior two thirds of the thalamus, a study of microtopographic

sections showed that the tumor mass represented essentially an enlarged subependymal cell deposit surrounding the vena terminalis and its tributaries and displaced the thalamus caudad without disrupting its normal internal structure and nuclear arrangement (fig. 23). The tumor extended also into the subependymal layer of the caudate nucleus within the anterior horn of the lateral ventricle. There was but a very small amount of invasion, and that was related to adjacent periventricular parts of the caudate nucleus and to the knee of the internal capsule. As a whole the main tumor was merely wedged in between slightly compressed but otherwise normal structures. Rostrally, and adjacent to the main tumor mass, there were subependymal granulations in the anterior horn of the lateral ventricle. These granulations consisted of small spongioblastic cell collections, closely resembling those noted in the tumor proper. The latter showed marked polymorphism, displayed by the presence of varying cell forms in different, though contiguous, regions. Thus, in one part there were elongated spongioblasts; in another large neuronal elements prevailed. An area near the terminal vein showed a large number of psammoma-like bodies, necrosis and extravasation. Of special interest was an area showing a great concentration of large neuronal elements. It protruded into the ventricle and was definitely not related to any normal brain structure containing nerve cells. This definitely established the neuronal elements as neoplastic (or blastomatous) and free of contamination with normal neuronal elements.

Case 8 illustrates the occurrence of multiple striatothalamic tumors of the spongioneuroblastic variety with one main focus and several smaller ones in the same or the opposite hemisphere. Besides the obvious relationship to the sulcus terminalis, it is to be noted that multiple subependymal nodules, often described as granulations, were present in this case, and had already been noted by Globus [15] and the Werthams [16] in connection with tuberous sclerosis.

Case 9 (Spongioneuroblastoma).—The patient, a woman aged 52, had been operated on for appendicitis nine years previously; a second operation was necessary one year later because of abdominal complications, and a long convalescence of over one year followed. Six months before her last admission to the hospital she was involved in an automobile accident without serious injury, an arm being bruised; no concussion was reported at that time. Four months later she began to suffer from severe headaches and displayed mental changes, with loss of memory, confusion and melancholia. In addition she complained of bodily weakness, hypersomnolence, constipation and nocturnal incontinence of urine. Transitory jaundice had occurred at the onset of these symptoms.

On admission the patient showed impaired memory and disorientation. The deep reflexes were hyperactive and the lower abdominal reflexes absent. Traces of albumin and pus cells were found in the urine. The blood count showed 10,500 white cells, 84 per cent of which were polymorphonuclear leukocytes and 16 per cent lymphocytes. The temperature was 102 F.; the pulse rate, 100. After an initial drop in the temperature to 100.6 F., the temperature rose slowly but steadily to 105.6 F. in the course of thirty-three days. The white blood cells increased to

15. Globus, J. H.: Malformations in the Central Nervous System (Tuberous Sclerosis), in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 1150.

16. Wertham, Frederic, and Wertham, Florence: The Brain as an Organ, New York, The Macmillan Company, 1934.

19,500. A tentative diagnosis of encephalitis, infection of the genitourinary tract or cholangitis was made.

On the thirty-third day, with the patient under local anesthesia, laparotomy was performed, but nothing abnormal was found in the abdomen. The patient's

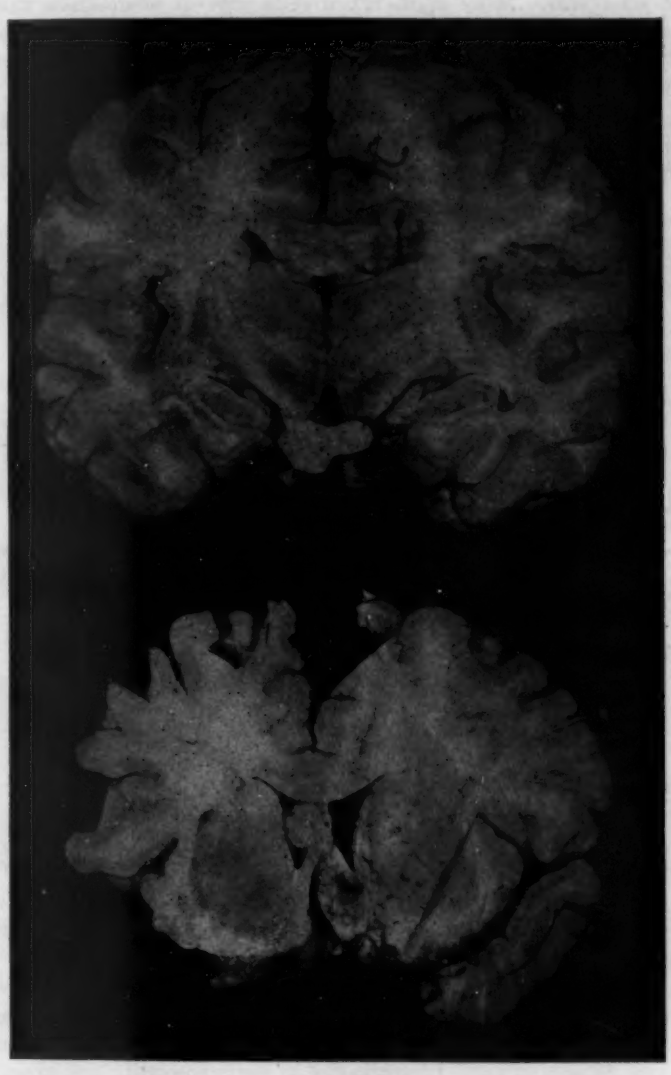

Fig. 24.—Coronal sections in case 9 (spongioneuroblastoma disseminatum), showing nodules in the septum pellucidum and one in the corpus callosum.

temperature rose to 108 F. after the operation; the pulse became rapid and imperceptible and the respirations rapid and shallow. The patient did not respond to stimulation and died twenty-four hours later. The autopsy disclosed a cerebral neoplasm.

Microscopic Anatomy.—The tumor tissue was densely cellular and well vascularized, with large areas of degeneration. The border zones of the tumor showed the character of infiltrating growth. The cellular elements consisted of irregularly shaped spongioblasts with scattered neuroblasts and numerous multinucleated giant cells. Many of the giant cells were of neuroblastic character, showing tigroid substance. Other giant cells belonged to the spongioblastic type. Mitotic figures were frequent. There was also a tendency to pseudorosette formation.

Gross Anatomy.—The brain was rather small with somewhat atrophic gyri. A nodule the size of a large kidney bean protruded from the wall of the septum pellucidum into the anterior horn of the left lateral ventricle. It was continuous

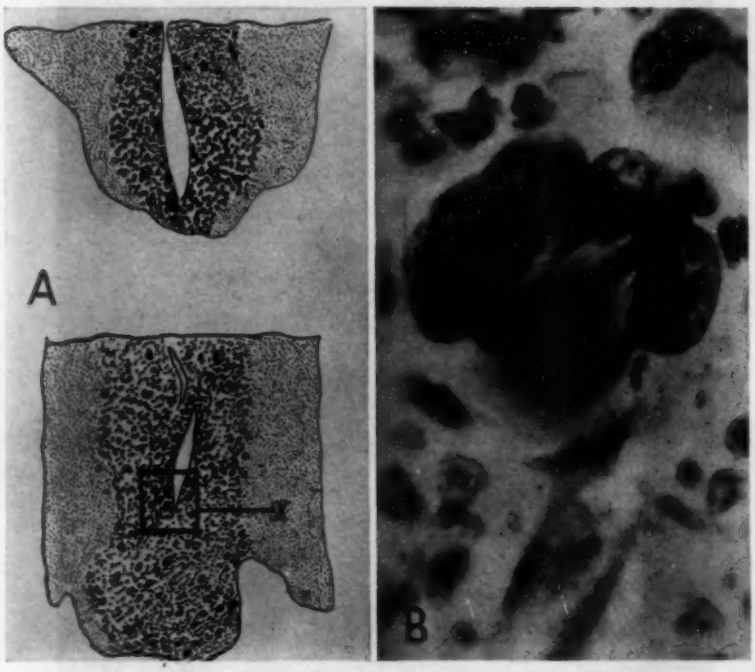

Fig. 25.—*A*, camera lucida drawing showing the distribution of the tumor about the third ventricle in case 9 (spongioneuroblastoma disseminatum) (×650). *B*, giant cells, one of many in the area marked by *X* in *A*.

with a mass of fairly firm consistency infiltrating the septum pellucidum and reaching the corpus callosum (fig. 24). The body of the corpus callosum was involved to a distance of about 1.5 cm. caudal to the interventricular foramen. The tumor mass extended into the right basal part of the septum pellucidum, the right nucleus accumbens and the region of fetal coalescence of the right anterior horn, ventral to the head of the caudate nucleus. There was a slight degree of dilatation of the right anterior horn of the lateral ventricle with narrowing and shifting to the left of the left anterior horn.

Another tumor was found in the hypothalamic region (fig. 25) directly dorsal and posterior to the chiasm, involving the subependymal cell plate of the third ventricle

on both sides of the midline. This tumor mass was of gray color and firm consistency and extended caudad for a distance of about 1 cm. It had destroyed the paraventricular nucleus in the preoptic and tuberal region and also the nuclei tuberis.

From the anatomic point of view the significant feature in case 9 is the multiplicity of the lesions with their distribution in zones in which residues of the subependymal plate are more commonly encountered. Thus, tumor nodules were found in the subependymal plate in the region of the septum pellucidum, in the coalescent zone of the anterior horn of the lateral ventricle and in the hypothalamus in the region of the tuber cinereum.

Clinical points of interest are the fact that the diagnosis during life was exceedingly difficult because of the atypical manifestations, such as the elevated temperature and the abdominal symptoms, all of which tended to lead one in search of some systemic disease. It is known, as already reported previously by Strauss and Globus,[17] that a lesion in the region of the tuber cinereum and the paraventricular nucleus of the optic region serves as a disturbing factor in the regulation of the temperature. In some instances the temperature becomes elevated, and in some instances it drops below normal. At any rate there develops a rather significant discord between pulse and temperature; the pulse drops while the temperature rises and vice versa.

Case 10 (Spongioneuroblastoma).—A man aged 42 had severe frontal headache for three days, accompanied by vomiting and a rise in temperature; shortly afterward he experienced a "bad odor" and repeated vomiting; one week later the headaches returned and were accompanied by impairment of vision, dizziness and pain in the right leg; transient episodes of confusion began at this time, accompanied by general weakness and apathy, and were followed by disorientation during the last week before admission.

The findings on admission were: restlessness and confusion; motor and sensory aphasia; complete amaurosis; bilateral papilledema with hemorrhages and exudates; pupils fixed to light; signs of bilateral involvement of the pyramidal tracts, more marked on the right side. The diagnosis was: a neoplasm in the left hemisphere invading the corpus callosum.

Terminal events were: a steady decline; conjugate deviation of the eyes to the left; terminal pyrexia; death on the sixth hospital day. There was no surgical intervention.

Microscopic Anatomy.—The tumor consisted of spongioblasts, most of which were radially arranged. Fat stains disclosed large amounts of fat globules within these cells. Multinuclear giant cells were many and showed unusually long processes (fig. 26) in contradistinction to those characterizing the giant cells in spongioblastoma multiforme. In various parts of the cerebral cortex there were heterotopic areas such as are found in tuberous sclerosis. Notwithstanding the spongioblastic character of its cells, the tumor was designated as spongioneuroblastoma, for it was

17. Strauss, I., and Globus, J. H.: Arch. Neurol. & Psychiat. **25**:506, 1931.

accompanied by a dissemination of lesions of tuberous sclerosis containing neural elements. Hence, the main tumor mass was regarded as a local intensification of disseminated spongioneuroblastosis, a term descriptive of the lesions in tuberous sclerosis.

Gross Anatomy.—The tumor involved the caudal part of the sulcus terminalis in the region of the left pulvinar on a level with the junction of the inferior and posterior horns of the lateral ventricle. It invaded the left occipital lobe as well as the adjacent deep parts of the parietal lobe in the vicinity of the parieto-occipital fissure.

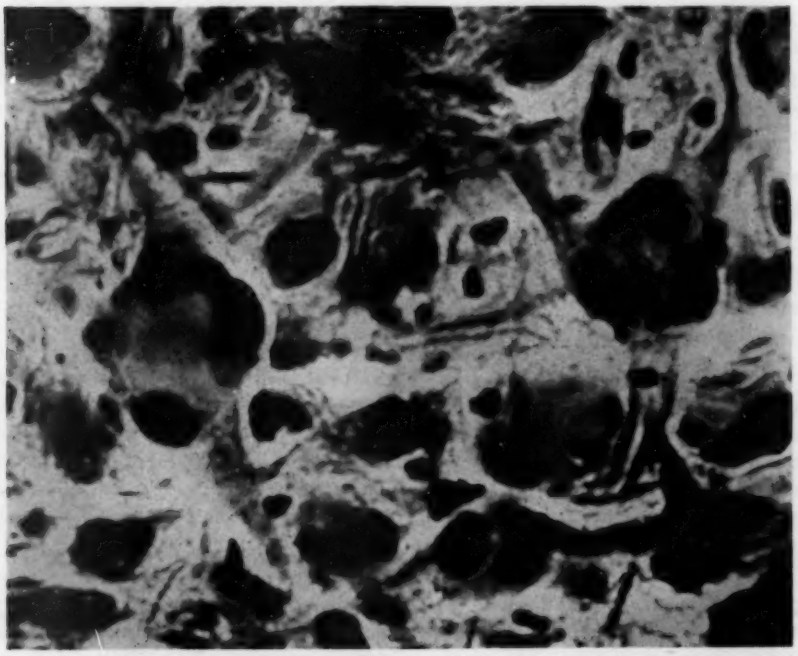

Fig. 26.—Giant cells with long processes in case 10 (spongioneuroblastoma) (×200).

In this as in other cases already described there was a close relationship between the growth and the sulcus terminalis. What is also quite significant is the finding of disseminated spongioneuroblastic areas in other parts of the brain, pointing to a developmental (blastomatous) anomaly as the underlying condition. Note that the location of the tumor was in an area which frequently is the seat of subependymal granulations as seen in tuberous sclerosis (case 12).

Case 11 (Spongioneuroblastoma).—A girl aged 15 experienced an abrupt onset of headache, vomiting and abdominal pain nine weeks before admission; five weeks

later squint of the right eye was followed by double vision; in another week difficulty in walking and tremor of the right hand set in; two days before entrance to the hospital, two convulsive clonic seizures occurred, and lapse into coma one day later.

The findings on admission were: coma; rigidity of the right arm; marked bilateral papilledema; hyperreflexia on the right side; a Babinski sign on the right; cerebrospinal fluid under high pressure. The diagnosis was: cerebral neoplasm.

Subtemporal decompression was immediately performed on the right side. The patient showed a rapid decline, with death occurring eleven hours after operation.

Fig. 27.—Coronal sections of the brain showing the gross appearance and the location of the tumor in case 11 (spongioneuroblastoma).

Microscopic Anatomy.—The tumor was densely cellular and consisted of a central and a peripheral zone, each showing somewhat different characteristics. In the central part, which was richly vascular and contained numerous necrotic areas, the cells were unipolar, bipolar and irregularly multipolar spongioblasts of various sizes. Multinuclear giant spongioblasts were frequent, and mitotic figures were present. There were also cells with scant cytoplasm, which can be interpreted as those representing a very immature form and in a sense the bipotential mother

cells of both the spongioblasts and the neuroblasts. The peripheral zone consisted of densely arranged fusiform cells streaming out in low rows and whorls. Some of these elongated cells were definitely unipolar and bipolar, while others had many short branched processes. Occasionally nerve fibers were seen lined by cell chains which resembled primitive interfascicular glia. The general histologic pattern of this zone corresponded to the type described as central neurinoma by Josephy [18] and spongioblastoma unipolare by Bailey and Cushing.[19] Histiotypic and heterotypic lesions of tuberous sclerosis were found in other parts of the cerebral cortex. Although the predominant tumor cells were essentially of a spongioblastic nature, the tumor, in view of the presence of tuberous nodules in the cerebral cortex, is designated as spongioneuroblastoma (see case 5).

Gross Anatomy.—The anterior part of the left thalamus was infiltrated by a tumor mass reaching as far dorsolaterally as the sulcus terminalis. An enlarged left thalamus protruded across the midline, compressing the third ventricle (fig. 27), the right diencephalon and the lenticular nucleus. A cystic cavity of walnut size was located in the posterior region of the left thalamus. The internal capsule and the lenticular nucleus on the affected left side were pushed laterally but without reduction in size or disorganization. The left lateral ventricle in this region was pushed upward and compressed. In the other parts of the ventricular system there was a moderate degree of internal hydrocephalus.

In the case just reported the relationship of the tumor to the sulcus terminalis is quite obvious.

Case 12 (Spongioneuroblastosis disseminata [tuberous sclerosis]).—The patient was a man aged 22. Two years before admission to the hospital he became subject to transient attacks of blurred vision; two months later there began a six week period of recurrent attacks of pain in the left eye, associated with hemorrhage in that eye; six months later there was a four week period of frontal headaches and blurred vision, with inward deviation of the left eye; one year later headache and double vision reappeared; at this time, there was paresthesia of the face, more marked on the left side, of one month's duration. During the last two weeks there were flattening of the left side of the face, pain in the right lumbar region and difficulty in finding the right words for expression.

On admission the findings were: bilateral choked disks; fine nystagmus in all directions; atrophy of the left temporal and masseter muscles; right biceps reflex more active than the left. A ventriculogram indicated the presence of a tumor near the posterior part of the third ventricle.

Terminally there were progressive increase in papilledema, slight left ptosis, weakness of inward gaze of the left eye, exophthalmos, bilateral corneal hypesthesia, bilateral diminution in hearing and slight supranuclear paresis of the right side of the face, in rapid succession. Another ventriculogram showed a filling defect in the body of the right lateral ventricle. Craniotomy was performed in two stages, and death followed on the fourth day after the second operation.

Microscopic Anatomy.—The tumor consisted predominantly of large spindle-shaped spongioblasts. However, primitive bipotential neuroepithelial cells as well

18. Josephy, H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **93**:62, 1924.

19. Bailey, P., and Cushing, H.: A Classification of the Tumors of the Glioma Group on a Histogenetic Basis, Philadelphia, J. B. Lippincott Company, 1926, pp. 47, 49, 59, 61, 62, 74, 82 and 93.

as young neuroblasts and an occasional, fairly mature nerve cell could be detected. The fusiform and often cone-shaped tumor cells frequently contained more than one nucleus. Whorl formations around cystlike necrotic areas were found in many

Fig. 28.—Longitudinal section of the hemisphere, showing the appearance and the location of residual portions of the tumor in case 12 (spongioneuroblastoma). Note the ependymal granulations.

places; psammoma-like concretions were very numerous. The tumor was associated with widely disseminated areas of tuberous sclerosis.

Gross Anatomy.—The body of the right lateral ventricle in its posterior two thirds was almost completely obliterated by a growth projecting from its lateral wall. In front of it, apparently corresponding to the region of the sulcus terminalis, there was an area of somewhat disorganized tissue, probably the seat of that part of the neoplasm which was removed by the operation. The right posterior and descending horns of the lateral ventricle were more distended than the corresponding parts of the opposite side. With exception of the part of the ventricle blocked by the neoplasm, the entire ventricular system was dilated. The ependymal lining of the ventricles was much thickened and presented an irregular granulated surface (fig. 28). A similar condition was present in the lining of the fourth ventricle. In several regions of the cerebral hemispheres there were marked retractions of the gyri, and these showed increased density on palpation.

This is another instance of striatothalamic tumor associated with well developed lesions of tuberous sclerosis in which subependymal granulations, probably derivatives of the subependymal plate, are very prominent. The relationship between the tumor and the sulcus terminalis could be traced.

Case 13 (Spongioneuroblastoma).—The patient was a woman aged 42. Five years before admission there developed weakness of the right foot; six weeks before admission there was weakness of the right arm with cramplike pain in the right arm and leg; four weeks later weakness of the right side of the face and impairment of speech were noted.

The findings on admission were: emotional instability; a mixed type of aphasia; right temporal hemianopia; complete right hemiparesis; findings on the right referable to the pyramidal tracts; impairment of all sensation on the right; hyperaffectivity on stimulation on the right. The diagnosis was: a right temporoparietal subcortical neoplasm.

An encephalographic examination was made. Stupor developed on the following day, and death occurred four days later. There was no surgical intervention.

Microscopic Anatomy.—The neoplasm was densely cellular and highly vascular, with areas of extensive necrosis. The cellular elements consisted mainly of spongioblasts of very irregular shapes. Multinuclear giant cells of polymorphous outline predominated (fig. 29). Some atypical monster cells could be recognized as neuronic elements. A number of vessels showed periadventitial condensations of primitive neuroectodermal elements. Mitotic figures were numerous.

Gross Anatomy.—The tumor was large with an approximate diameter of 4 to 4.5 cm. and was situated within the left hemisphere, on a level with the island of Reil. It was invasive and extended from the anterior third of the sulcus terminalis through the posterior limb of the internal capsule, the posterior part of the lenticular nucleus and the entire thalamus into the basis pedunculi medial to the optic tract. In the thalamus only a narrow mesial strip adjoining the third ventricle had escaped destruction.

Attention may be drawn to the peculiar type of giant cells, which bore evidence of their neuroblastic lineage. The large number of mitotic figures is a feature often encountered in tumors of the spongioneuroblastic variety. The relationship to the sulcus terminalis is quite obvious.

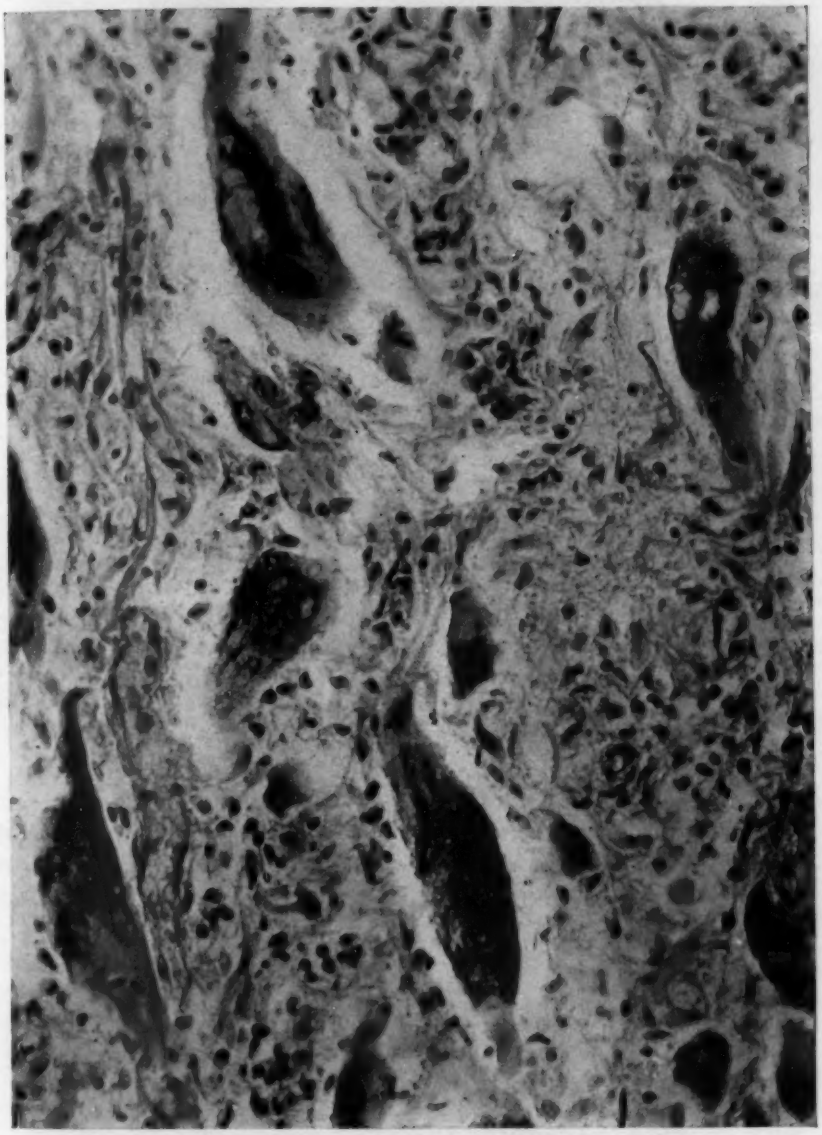

Fig. 29.—Giant cells of an unusual variety in the tumor in case 13 (spongioneuroblastoma) (× 408).

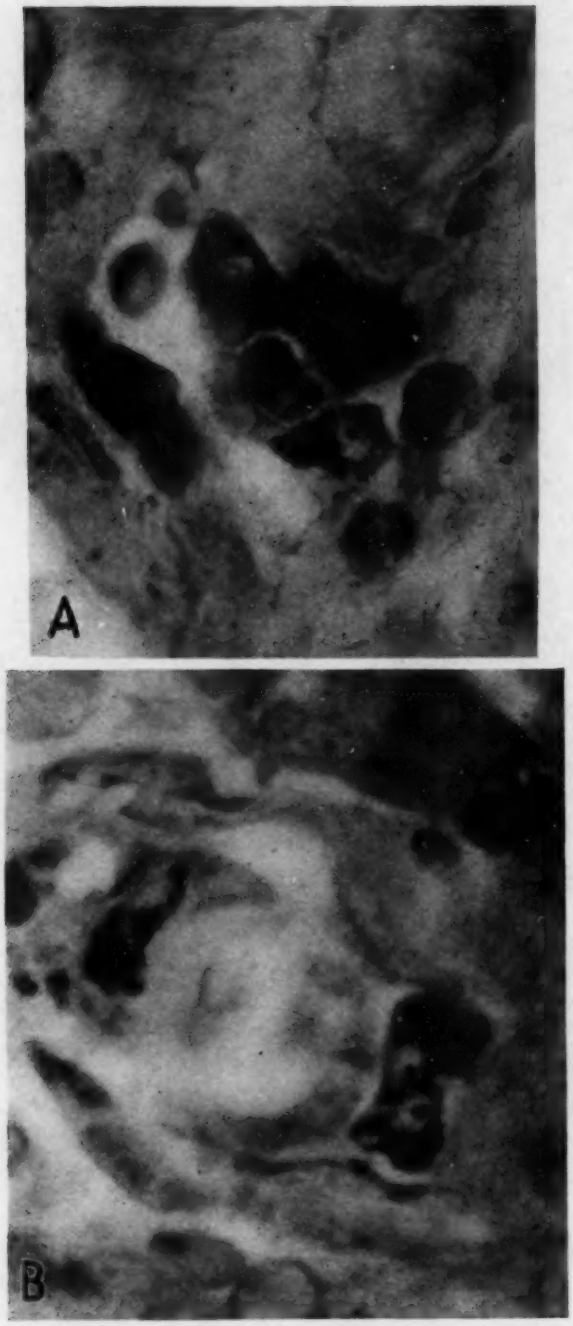

Fig. 30.—Maturing neuroblasts in the proximity of blood vessels in case 14 (spongioneuroblastoma) (Nissl preparations; *A*, × 1,450; *B*, × 1,375).

CASE 14 (Spongioneuroblastoma).—The patient was a man aged 40. Six weeks before admission he experienced the onset of drooping of the left side of the mouth; one week later he suffered from right-sided headaches and weakness of the left upper extremity and in another week he showed euphoria and inattention.

The findings on admission were: bilateral papilledema, greater on the left side; mimetic weakness on the left side of the face; a bilateral Kernig sign; upper deep reflexes more active on the left; lower deep reflexes more active on the right; weakness and poor coordination in the left upper extremity. The diagnosis was: a neoplasm of the right frontal-parietal region.

Craniotomy was done. There was a rise in temperature; a gradual decline began and death occurred twenty-seven days after operation.

Microscopic Anatomy.—The tumor was densely cellular and well vascularized. There were many areas of necrosis and extravasation. The tumor cells were predominantly spongioblasts displaying great variation in their size and shape. Surrounding areas of necrosis, the cells were arranged in palisades and rosettes. Numerous multinuclear giant cells and many mitotic figures were seen. Nissl preparations revealed the presence of numerous nerve cells in various stages of maturation. Often they were found in the proximity of blood vessels or in small nests; occasionally such cells were multinuclear (fig. 30). The blood vessels showed hypertrophy of intimal cells and marked thickening of the adventitia. Hyalinization of the vascular wall was found in various places.

Gross Anatomy.—The tumor projected into the anterior horn of the right lateral ventricle, at the base of the head of the caudate nucleus, and extended caudad as far as the interventricular foramen, thus connecting with the rostral end of the sulcus terminalis. The neoplasm had invaded the head of the caudate nucleus, extending laterally into the internal capsule and the lentiform nucleus, affecting particularly the globus pallidus. Caudally and ventrally it became continuous with a hemorrhagic area in the hypothalamic region. The latter, a large rounded mass, extended into the tegmentum of the right side of the midbrain. A linear midline hemorrhage could be traced through the mesencephalon into the pons.

Little need be said about the quite evident relationship of the neoplastic process to the rostral end of the sulcus terminalis. What is particularly striking is the neuroblastic preponderance of cellular make-up of the tumor. There is no question that the nerve cells even in their more mature forms were of neoplastic origin.

CASE 15 (Spongioneuroblastoma).—A woman aged 66 had been subject to recurrent headaches for thirty-four years; she was known to have been hypertensive for nine years. The diagnosis on her first admission to a hospital was cerebral arteriosclerosis. Two years before her final admission she experienced sudden onset of dizziness and inability to walk, followed by memory changes and somnolence. An episode of confusion followed by coma of three days' duration led to her second admission to the hospital.

On admission she was in a deep sleep. The right palpebral fissure was larger than the left, and there were grasping and sucking reflexes. The diagnosis was: a vascular lesion, probably in the hypothalamus close to the sleep-regulating center.

Terminally there were a rise in temperature to 107.6 F. and a progressive decline with pneumonia, followed by death nine days after the onset of the deep sleep. There was no surgical intervention.

Microscopic Anatomy.—The tumor was densely cellular and very vascular. The bulk of the neoplasm was formed by round, pear-shaped, elongated or irregular cells of neuroblastic character (fig. 31 *A*), revealing tigroid substance in Nissl

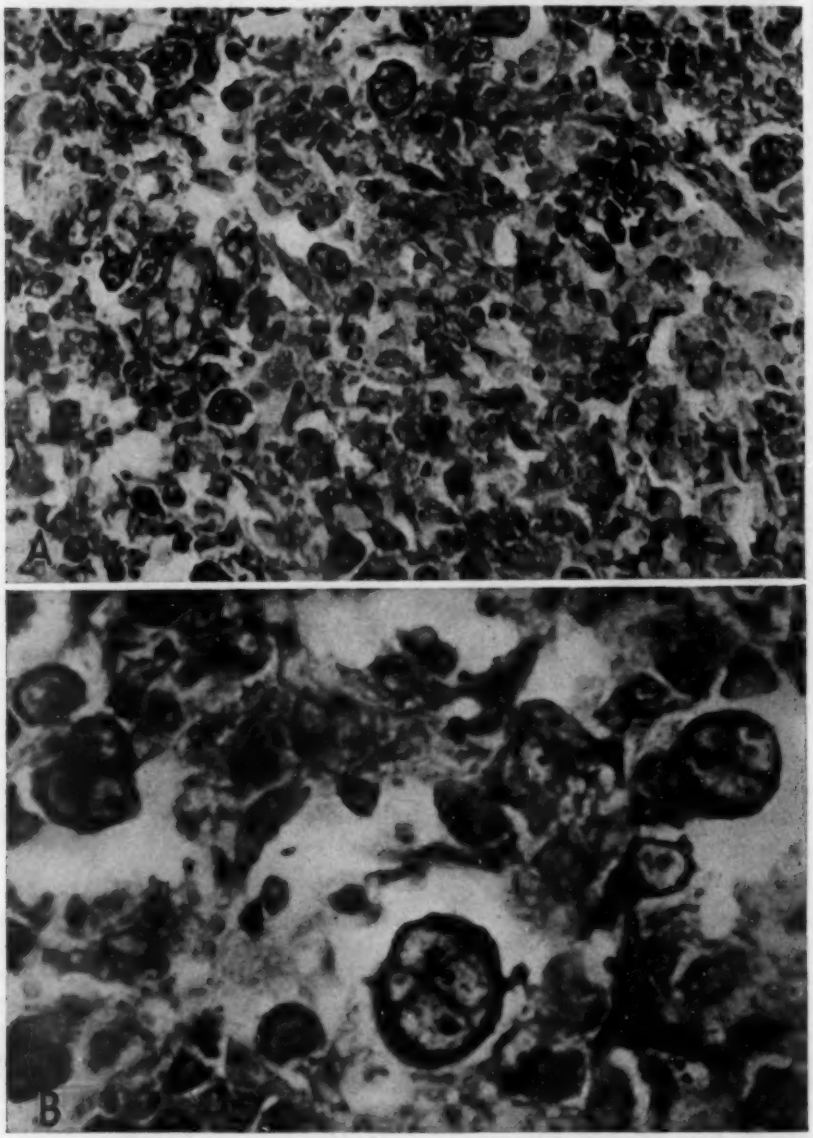

Fig. 31.—*A*, histologic character of the tumor in case 15 (spongioneuroblastoma), showing many multinuclear neuroblasts (Nissl stain; × 300). *B*, giant multinuclear neuroblasts (Nissl stain; × 835).

preparations. Many of the larger cells were multinucleated neuroblasts (fig. 31 *B*). Besides these cells there were many smaller undifferentiated elements with round

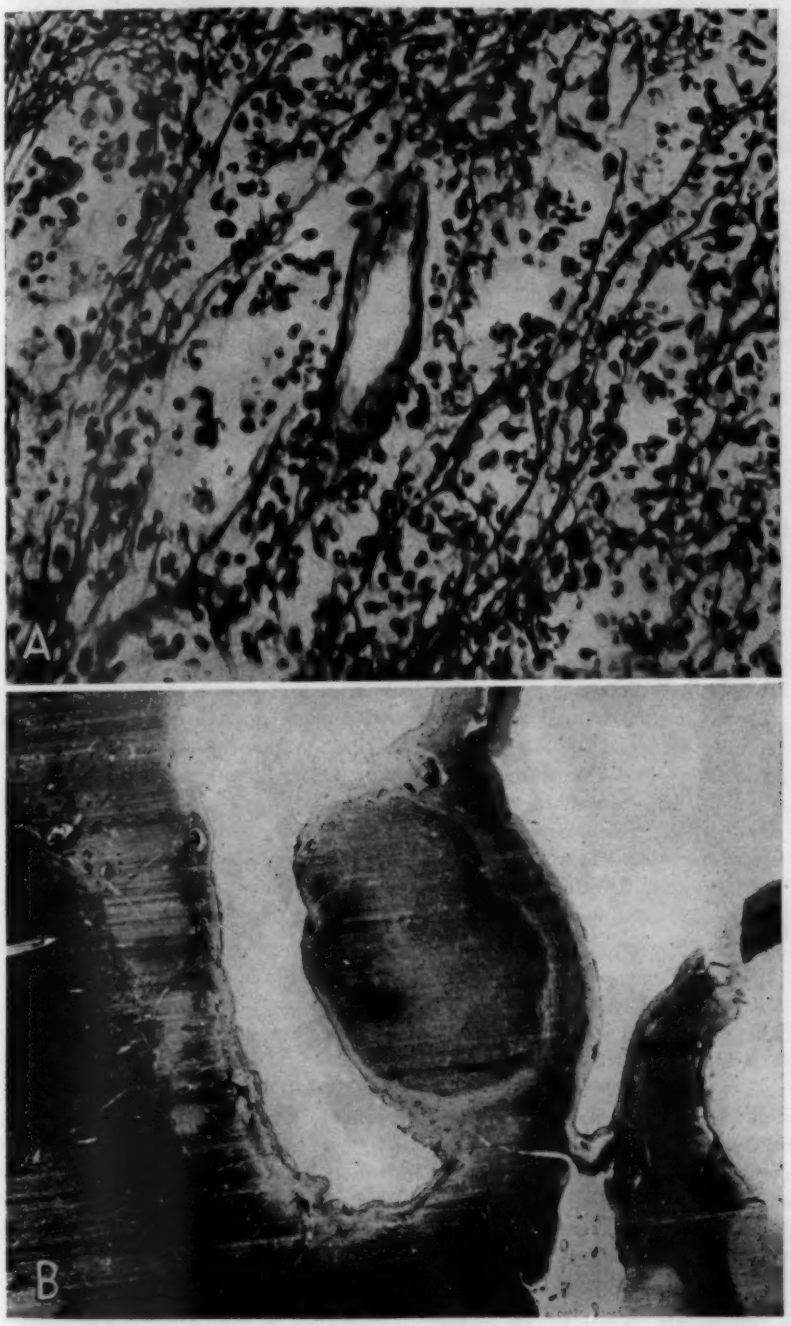

Fig. 32.—*A*, reticular fibers in the stroma of the tumor in case 15 (spongioneuroblastoma) (silver stain, × 390). *B*, coronal section showing the same tumor in the septum pellucidum (× 10).

nuclei and unstained protoplasm, also some fusiform spongioblastic forms. Mitotic figures were numerous. There were thin-walled vessels and others with thickened intima and adventitia. Many of the vessels were accompanied by periadventitial rows and condensations of smaller cells. The adventitial reticular fibers of the blood vessels extended into the tumor, forming a very dense network of delicate lattice fibers throughout the neoplasm (fig. 32 *A*).

Gross Anatomy.—The neoplasm was rather small, measuring less than 2 cm. in diameter. It originated from the subependymal layer of the septum pellucidum and projected into the right lateral ventricle. This layer, as was mentioned earlier, is one of the sources of embryonal rests. The neoplasm was of limited invasive character and well delineated in the right septum (fig. 32 *B*), protruding into the right lateral ventricle and reaching the ipsilateral fornix at the latter's point of entrance into the thalamus. The medullated fiber bundles running in the wall of the affected septum pellucidum and belonging to the fornix system were neither degenerated nor compressed. The cavum septi was markedly distended. Both lateral ventricles were enlarged, but the right more than the left.

This tumor was one of several in our series showing definitely its relationship to the subependymal cell plate in the septum pellucidum. Moreover, it was an outspoken example of the spongioneuroblastoma in which the neuroblastic elements are in a preponderant ratio. In addition it was rich in unusual forms of multinucleated neuroblasts.

Case 16 (Spongioneuroblastoma).—Five months before admission to the hospital a man aged 24 experienced the onset of impairment of vision in the left eye; six weeks later there was progressive disturbance of vision in the right eye.

The findings on admission were: marked diminution of vision, especially in the right eye; bilateral papilledema, more marked on the right side; cerebrospinal fluid under an initial pressure of 280 mm. of water. The diagnosis was: a neoplasm at the base of the brain near the optic chiasm.

Transfrontal craniotomy was done on the right side; herniation of the brain was noted at operation. There was residual left hemiparesis, with complete amaurosis one month later. Death occurred two and a half months after operation.

Microscopic Anatomy.—The tumor consisted of a central densely cellular neoplastic part alternating with small necrotic areas containing macrophages filled with large amounts of blood pigment. The tumor cells in this part were elongated spindle-shaped or carrot-shaped spongioblasts with a fair amount of cytoplasm. These cells varied greatly in size and gave rise to numerous well stained processes, which formed streamlike patterns. Multinucleated spongioblasts were frequent. Many blood vessels were accompanied by periadventitial rows of primitive neuroectodermal elements (fig. 33 *A*), as previously described[1] for spongioneuroblastoma and some forms of glioneuroma. The outer zone of the tumor was less dense and contained spongioblasts as well as neuroblasts and other multipolar, atypical neuronic elements. The outer zone showed also perivascular cell aggregations. There the perivascular cells, however, were small round cells, apparently mesodermal in derivation (lymphocytes).

Gross Anatomy.—In the region of the right basal ganglions and thalamus near the rostral end of the sulcus terminalis there was a firm grayish tumor mass encroaching on the opposite ventricle and deforming the septum pellucidum. The

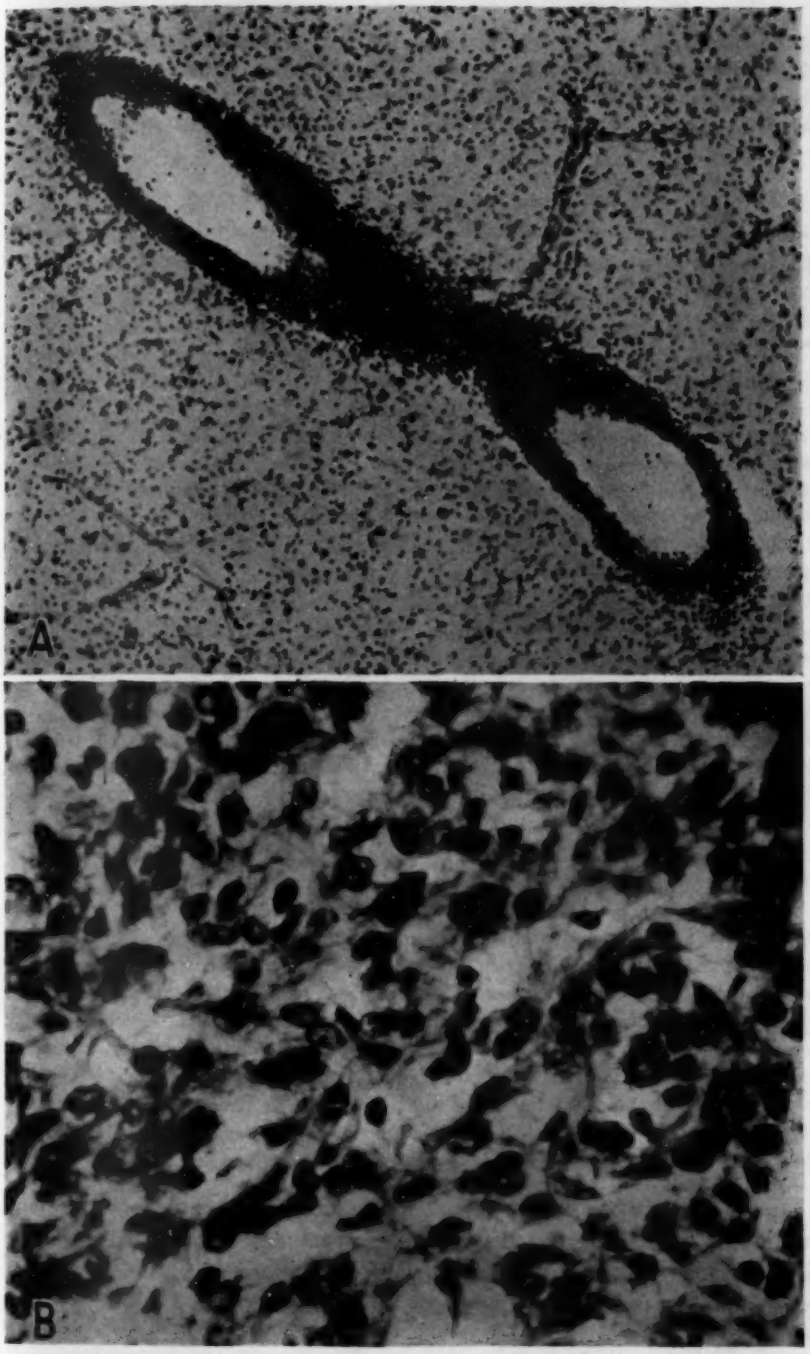

Fig. 33.—*A*, perivascular aggregation of cells in case 16 (spongioneuroblastoma) (×100). *B*, histologic appearance of the tumor in case 17 (transitional glioma) (×585).

tumor mass appeared well delimited from the surrounding brain tissue, which was disorganized and in places hemorrhagic. There was also softening of brain tissue between the tumor and the hernia. The posterior horn of the right ventricle was greatly enlarged and contained viscid, jelly-like colloid material.

In the case just described, aside from the close relationship of the tumor to the sulcus terminalis, attention may be drawn to the accumulation of cells about blood vessels, which is frequently encountered in tumors of the spongioneuroblastoma category.

CASE 17 (Transitional glioma).—A woman aged 40 had been irritable for two years before admission to the hospital; there had been moderate blurring of vision for one year, marked personality changes of three months' duration, frontal headaches during the last two weeks, accompanied by somnolence, increased by loss of vision, and followed by right-sided hemiparesis.

The findings on admission were: moderate bilateral papilledema; right-sided signs of involvement of the pyramidal tracts; decrease of all forms of sensation on the right; cerebrospinal fluid under initial pressure of 240 mm. of water. The diagnosis was: a neoplasm in the left frontoparietal region.

Seven days later there occurred sudden coma, followed by death within two hours, with no surgical intervention.

Microscopic Anatomy.—The neoplasm was densely cellular and moderately vascular, sharply circumscribed in some places and infiltrating in others. There were fusiform and irregular spongioblastic cells besides densely crowded cells with ovoid nuclei and little cytoplasm (fig. 33 *B*). Numerous mitoses could be seen. Extravasation and in one place some concretions were present. The more loosely arranged cells were more differentiated and could be recognized as fairly mature astrocytes. Here and there a few multinucleated cells might be seen; they did not assume large dimensions. Occasional elements with a well defined, centrally located nucleolus suggested the possibility of neuroblastic differentiation. The Nissl stain, however, failed to yield more definite proof of their neuroblastic character.

Gross Anatomy.—The tumor, a rounded mass about 4 cm. in diameter, had invaded the posterior half of the left thalamus by infiltration. It compressed the adjacent caudal part of the posterior limb of the internal capsule and the pes pedunculi. By its caudal extension the neoplasm had involved the pulvinar and the medial geniculate body. With the compression of the mesencephalon, the aqueduct had become a narrow dorsoventral slit. The tumor mass was connected with the sulcus terminalis along the caudal half of this structure. The third ventricle was displaced to the right and reduced to a narrow cleft. The body of the left lateral ventricle was displaced upward and had also become narrow. The right lateral ventricle was moderately enlarged.

The relationship of the tumor in the foregoing case to the sulcus terminalis has been pointed out in the gross description. This is the first instance in our group of a transitional form of glioma, a tumor type which is potentially malignant, not remote from spongioblastoma multiforme.

CASE 18 (Transitional glioma).—A man aged 65 had undergone three generalized convulsions during the three days before entering the hospital.

The findings on admission were: periods of semistupor; aphasia during lucid periods; conjugate deviation of the eyes to the left; weakness of the right extremities; slightly increased deep reflexes and absence of abdominal reflexes on the right side; rigidity of the neck and a positive bilateral Kernig sign; cerebrospinal fluid under initial pressure of 420 mm. of water, the fluid somewhat xanthochromic. The diagnosis was: anterior cerebral thrombosis on the left.

Terminally there was pulmonary edema, with death occurring on the third hospital day, with no surgical intervention.

Microscopic Anatomy.—The tumor was of moderate cellular density, infiltrating in character. It was well vascularized and showed extensive extravasations. Many elements were of a more primitive, spongioblastic type; others were more differentiated and appeared in Cajal preparations as fairly mature astrocytes. Multinuclear elements were scarce and were of small dimensions. This tumor was more advanced in its histologic differentiation than the preceding one.

Gross Anatomy.—The tumor was found in an area of red softening about 5 cm. in length and 4 cm. in width, destroying the left caudate nucleus, the anterior limb of the internal capsule and the anterior extremity of the thalamus. The rostral end of the sulcus terminalis and the subependymal cell plate in the rostral part of the head of the caudate nucleus in this instance were in a definite and readily demonstrable connection with the neoplasm. The frontal part of the corpus callosum showed softening and discoloration of the white matter on the affected side, and the frontal pole showed a similar disorganization.

In this instance a definite continuity was found between the subependymal cell plate in the vicinity of the head of the caudate nucleus and the tumor tissue.

Case 19 (Glioneuroma).—Nine months before admission to the hospital a woman aged 41 complained of dizziness, nausea and hot flushes; two months later generalized convulsions occurred at the date when menstruation would have taken place (she had undergone hysterectomy for fibroids). During the next six months she suffered from dizziness, somnolence, headache and falling to the left, progressing in severity.

The findings on admission were: inability to sit up; bilateral papilledema; nystagmus, more marked to the right; dysdiadokokinesis on the left; depression of deep reflexes; a Babinski sign on the right; cerebrospinal fluid under increased initial pressure. The diagnosis was: a neoplasm, probably in the posterior fossa.

A ventriculographic examination was made three days later; transient visual hallucinations set in and stupor followed, ending in death. There was no surgical intervention.

Microscopic Anatomy.—The tumor was densely cellular and moderately vascularized. The blood vessels showed fairly thick walls. Many giant cells were present. Most of the elements, however, were of an immature glial variety, suggesting a transitional form. In Nissl preparations there were many neuroblastic cells in various stages of maturity. Mitotic figures were few.

Gross Anatomy.—The cut surface of the tumor had a gelatinous appearance. The growth was connected with the subependymal layer of the head of the right caudate nucleus, and by infiltration it involved the centrum semiovale of the right hemisphere, extending into the temporal lobe. The anterior horn of the lateral ventricle of the opposite side and the anterior part of the third ventricle were compressed (fig. 34).

Of interest in this case is the strong similarity of the location of the tumor to that of the tumor in case 7. The position of the tumor basal to the head of the caudate nucleus suggests a relationship to the former

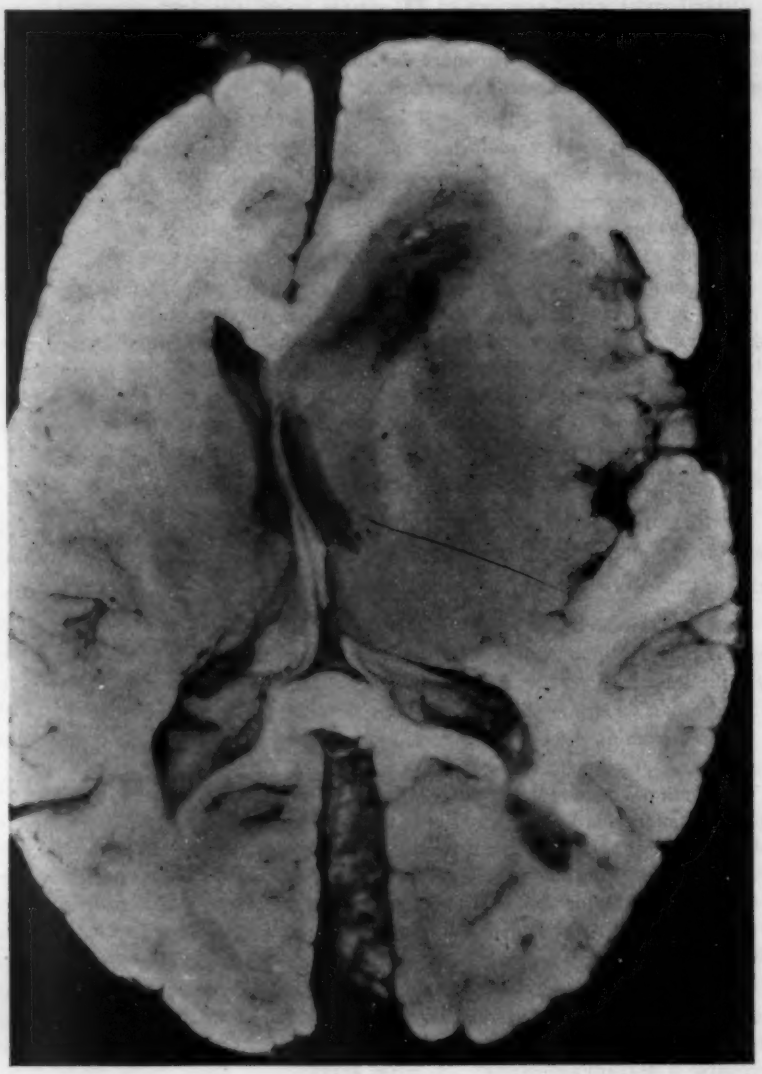

Fig. 34.—Gross appearance and location of the tumor in case 19 (glioneuroma).

ventricular extension into the rhinencephalon, as was pointed out in our introduction and discussion of embryonic factors. Not without significance is the close correspondence of the cellular characteristics of these two tumors, the difference being mainly in the degree of maturity of the cellular forms.

CASE 20 (Glioneuroma).—Five weeks before admission to the hospital a woman aged 45 had gradual development of progressive fatigue and drowsiness; two weeks later she had severe headache, occasionally accompanied by vomiting.

The findings on admission were: drowsiness; unsteadiness of gait; left pupil larger than the right; both pupils fixed to light and reacting sluggishly in accommodation; slight supranuclear weakness of the left side of the face; poor coordination in the extremities; a suggestion of a Babinski sign on the left. The diagnosis rested between encephalitis and midbrain tumor. Three weeks after entry into the hospital, papilledema and a bilateral Babinski sign were noted, and projectile vomiting became frequent.

A ventriculogram suggested a lesion in the region of the right temporal horn of the lateral ventricle. Craniotomy was performed and a tumor found in the angle between the temporal horn and the body of the right lateral ventricle; death occurred one week postoperatively.

Microscopic Anatomy.—The tumor was well vascularized, with many extravasations, and macrophages crowded with blood pigment were frequent. The neoplasm was of fair cellular density, with areas of more loosely scattered cells. Some spongioblastic elements were elongated and arranged in parallel strands, suggesting the structure of the so-called "central neurinoma." A large quota of the cells, however, were of the more differentiated astrocyte type. There were many multipolar nerve cells and less differentiated neuroblasts (fig. 35*A*).

Gross Anatomy.—A large tumor mass was found involving the basal ganglions and the thalamus of the right hemisphere. The anterior limit of the neoplasm corresponded with the posterior limits of the insula. The dorsal boundary corresponded to the roof of the lateral ventricle. Medially the tumor approached the third ventricle, leaving only about 7 mm. of uninvolved tissue between it and the ventricular wall. Caudally the tumor involved very definitely the posterior part of the sulcus terminalis and reached the midbrain, causing a swelling of the tegmentum. The aqueduct was displaced dorsally and to the left; the substantia nigra was displaced ventrally. In the cerebral hemispheres and thalamus the tumor was hemorrhagic, granular and friable, while in the midbrain it was homogeneous and solid.

From the gross description the relationship of the tumor in case 20 to the posterior bend of the sulcus terminalis is quite obvious.

CASE 21 (Glioneuroma).—A man aged 36 had complained for eight years of general weakness, fatigability, episodes of semisleep-like states lasting hours at a time, and gastrointestinal disturbances. Four months before admission, severe nocturnal headaches and pain in the neck developed. Intense dizziness developed during the last four days.

On admission there were no signs indicative of focal organic disease; the cerebrospinal fluid was under an initial pressure of 240 mm. of water.

On the eighth hospital day there was a generalized convulsion, followed by stupor lasting ten minutes. An encephalographic examination was followed by coma, and death occurred within twelve hours. There was no surgical intervention.

Microscopic Anatomy.—The tumor consisted of many mature neurons, mostly small sized but well differentiated. They maintained a fairly regular distribution, although they were more crowded and lacked the arrangement of the normal nuclear masses. These cells lay in a matrix consisting of numerous immature glial elements of astrocytic variety. At the periphery of the frank tumor infiltra-

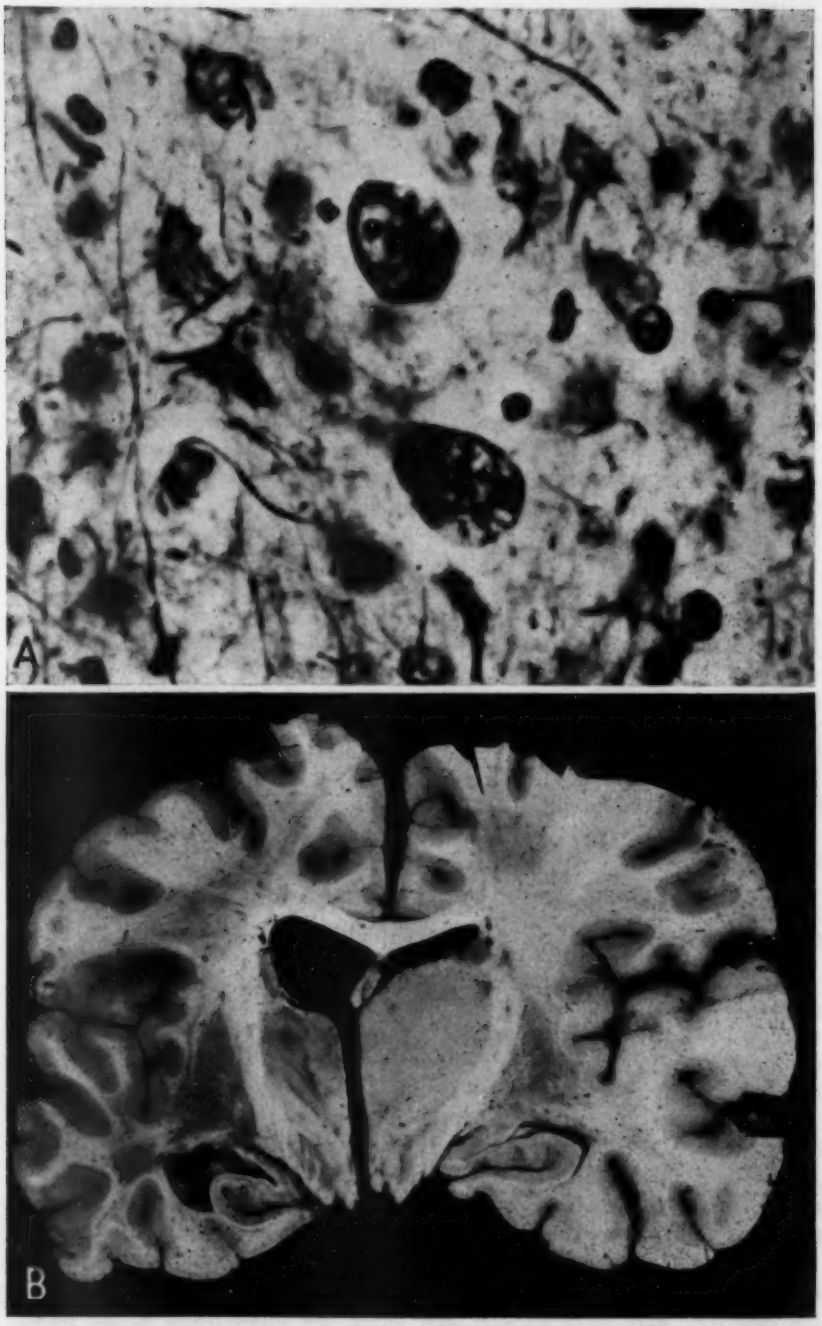

Fig. 35.—*A*, neuroblasts in an advanced stage of ripening, surrounded by fairly mature glial elements, in the tumor in case 20 (glioneuroma) (×940). *B*, gross appearance of the tumor in case 21 (glioneuroma).

tion there was an occasional giant nerve cell. Numerous degenerative changes, satellitosis and other anomalies related to tuberous sclerosis were also present. Many arachnoid cell clusters were noted in the meninges.

Gross Anatomy.—The entire right thalamus was markedly enlarged (fig. 35 *B*), but the enlarged thalamic nuclei retained recognizable boundary lines. Other areas of tumor formation were distributed throughout the entire right cerebral hemisphere. In the right superior temporal gyrus the gray matter was markedly reduced in volume. The left lateral ventricle was markedly dilated in all its subdivisions, while the right ventricle was only slightly enlarged.

In this case the neoplastic process in the thalamus assumed the character of hyperplasia without disturbing the internal structure of that portion of the brain. Because of the dissemination of the lesion it bore a strong similarity to tuberous sclerosis, which is often found associated with spongioneuroblastoma.

COMMENT

In the introduction we called attention to the zone of the striatothalamic junction which we have traced to the embryonal hemispheric stalk. It was also pointed out that its intimate relationship to the sulcus terminalis aids in its ready recognition and marks its extent. We have also emphasized the significance, the prominence and the wide distribution of the subependymal cell plate, for it, as well as the striatothalamic junction, in their entire extent are rich in embryonal residues, which may persist for variable lengths of time. Their presence is in harmony with the observations of Allen,[13] who found that the process of cell division in the brain of the rat is maintained longest in the paraependymal matrix in the regions of the caudate nucleus and the striatothalamic junction. Immature cells in the subependymal lining of the adult human brain were also found by Opalski.[11a] These residues by virtue of their inherent blastomatous potencies may act as a source of neoplastic processes and topographically serve as sites of predilection for tumor formation. Furthermore, these embryonal residues may constitute germinal centers from which any or all of the three neuroectodermal derivatives, i. e., the ependymal, glial and neuronal cell forms, may develop. Therefore, tumors arising from such embryonal rests will most likely contain cells of all these three varieties or else will contain a predominance of one type not, however, to the exclusion of other neuroectodermal cell forms. With this in mind, it can be readily seen why tumors in a series such as that presented are likely, because of their location, to be of the less differentiated, spongioneuroblastic variety, with spongioblastoma multiforme and ependymale next in frequency.

It is not without interest to note at this point that Smyth and Stern,[19a] studying cases of tumor of the thalamus, have offered a "hypothesis that the subependymal layer gives rise to the intrinsic gliomas of the thalamus."

19a. Smyth, G. E., and Stern, K.: Brain **61**:339, 1938.

To the zone of the striatothalamic junction and the subependymal cell plate we have added the zone of coalescence of the walls of the obliterating parts of the anterior horns of the lateral ventricles. Here too are found embryonal remnants capable of giving rise to neoplastic processes.

The relationship between these several zones, frequent sites of embryonal residues, and the tumor formations, is believed to have been demonstrated in the cases herein described, as shown in the accompanying table.

In line with the embryonal concept of tumor formation is the view that tumors take their origin from the least differentiated cell form. It may therefore be assumed that the growth of a tumor is predicated by the existence of primitive cell forms even in the so-called benign tumors which consist only of highly differentiated cell types (Globus [20]).

Tabulation of Presumable Sites of Origin of the Neoplasms in this Series

Sulcus terminalis	1,* 3, 4, 5, 6, 7, 8,*† 10,† 11,† 12,*† 13, 14, 16, 17, 20, 21
Coalesced portion of anterior horn	2, 7, 9,* 19
Subependymal cell plate, septum pellucidum	1,* 2, 6, 9,* 15
Subependymal cell plate, caudate nucleus	1,* 12,*† 18
Subependymal cell plate, hypothalamus	9*
Subependymal cell plate, recessus lateralis ventriculi IV	1*

* The numerals refer to the case numbers in the series. In cases distinguished by the asterisk the tumor was multiple.

† In cases distinguished by the dagger the neoplasm was associated with tuberous sclerosis.

With this in mind, it may be further postulated that tumors which consist of poorly differentiated cell forms of either neuroblastic or spongioblastic lineage are capable of rapid proliferation and growth, constituting the so-called malignant brain tumors, and that tumors which contain a large quota of highly differentiated cells are less rapidly growing and therefore less malignant. The prevalent view, accepting the existence of an absolutely benign neuroectodermal tumor in the brain, cannot be left unchallenged when it is realized that in order for the tumor to grow it requires cell groups of low differentiation and high growth potency. Here we find also the reason why we have not encountered among the tumors in this series any that could be regarded as truly benign glioma and why the ratio of the very malignant tumors among them as well as among other tumors of neuroectodermal derivation assembled in the Mount Sinai Hospital collection was rather high. In the accompanying diagram (fig. 36) we offer a schema which

20. Globus, J. H.: J. Mt. Sinai Hosp. **7**:361, 1941.

illustrates this relationship of the malignancy of a tumor to the stage of differentiation of its component cells and at the same time brings a simple classification of some of the primary brain tumors.

The term "spongioblastoma" was introduced as early as 1918 (Strauss and Globus [21]) and later, in 1925, was modified to read "spongioblastoma multiforme," [22] and was employed to designate a tumor of gliogenous character which consisted predominantly of primitive glial elements, the spongioblasts. It was then pointed out that, in addition to other features, giant multinucleated cells constitute the characteristic feature of such tumors. It was also then suggested that the large multinucleated giant cells typifying the spongioblastoma were probably an expression of rapid atypical cell division, which in turn

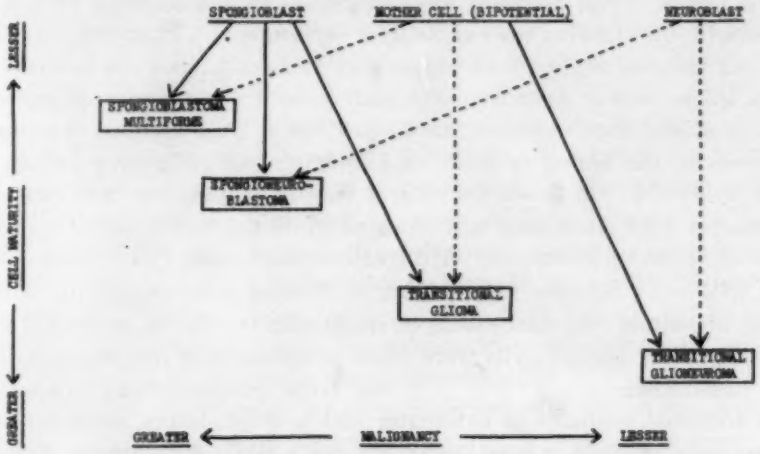

Fig. 36.—Diagram illustrating the interdependence of neuroectodermal derivatives and the histologic character of the related brain tumors. The solid line represents the probable main source of origin; the broken line, the possible additional source.

was believed to account for the rapid tumor growth. This view was contested by some (Bailey [23]; Elvidge, Penfield and Cone [24]), who considered these giant cells as degenerative forms prevailing in and about necrotic zones in the spongioblastoma. However, through sub-

21. Strauss, I., and Globus, J. H.: Neurol. Bull. **1**:273, 1918.

22. Globus, J. H., and Strauss, I.: Arch. Neurol. & Psychiat. **14**:139, 1925.

23. Bailey, P.: Primary Tumors of the Brain, in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 917.

24. Elvidge, A.; Penfield, W., and Cone, W.: The Gliomas of the Central Nervous System, A. Research Nerv. & Ment. Dis., Proc. (1935) **16**:107, 1937.

sequent observations, the fact was established that these giant cells are tumor cells occurring in the more malignant forms of primary brain tumors. Moreover, it was possible to demonstrate their reproduction in spongioblastoma grown in culture (Kredel [25]; Canti, Bland and Russell [26]).

As spongioblastoma was further studied,[27] it soon became apparent that many of the giant cells and some of the medium-sized cells were not glial in character, and with the employment of a very simple staining method, that of Nissl, it was soon established that many of the cells were of the neuroblastic type. Doubt again was expressed as to the existence of tumors in which the dominant cell forms were of the neuroblastic lineage and for which the terms "glioneuroma" and "spongioneuroblastoma" were suggested (Globus [28]; Farnell [29]). Bailey, who earlier [30] had accepted the views of Globus, concurred later with Penfield [31] in denying the existence of such tumors. However, a review of our material studied with satisfactory methods (Nissl; Bielschowsky) has led us to the conviction that such tumors (spongioneuroblastoma) do exist and that, moreover, they constitute a large number of primary tumors of the brain, as much as 12 per cent of gliogenous tumors in our collection. In this connection it may be pointed out that neuronal elements were also found and reported by Bailey and Cushing [32] in the so-called medulloblastoma. A direct quotation from their article reads as follows: "Schaper believed his indifferent cells capable of differentiation either into neuroblasts or spongioblasts. In one restricted area in one of our tumors, cells were found which showed the characteristics of neuroblasts. . . . " ". . . the large circular nuclei, containing no scattered granules of chromatin and a single heavy nucleolus surrounded by heavily stained cytoplasm, can scarcely be interpreted otherwise than as belonging to neuroblasts." "There can be no doubt, therefore, that although the majority of the neoplastic cells are potential neuroglia cells, some are potential neurones." Because of the presence of neural elements in these tumors, Roussy, Oberling and Raileanu [33] suggested the term "medulloblastoma neuromatosum" for them. Thus,

25. Kredel, F. E.: Arch. Surg. **18**:2008, 1929.

26. Canti, R. G.; Bland, J. O. W., and Russell, D. S.: A. Research Nerv. & Ment. Dis., Proc. (1935) **16**:1, 1937.

27. (*a*) Globus, J. H.; Strauss, I., and Selinsky, H.: Ztschr. f. d. ges. Neurol. u. Psychiat. **140**:1, 1932. (*b*) Globus, J. H.: Tr. Am. Neurol. A. **63**:35, 1937.

28. Globus (footnotes 1, 15 and 27 *b*).

29. Farnell, F. J.: Tr. Am. Neurol. A. **64**:28, 1938.

30. Bailey, P., in discussion on Globus.[27b]

31. Penfield, W., in discussion on Farnell.[29]

32. Bailey, P., and Cushing, H.: Arch. Neurol. & Psychiat. **14**:192, 1925.

33. Roussy, G.; Oberling, C., and Raileanu, C.: Presse méd. **39**:977, 1931.

the coexistence of both glial and neuronal elements was established in brain tumors of another variety. It may be accepted that conclusive evidence was presented here and elsewhere that there are primary tumors in the brain in which neuroblastic elements are present in variable numbers. Some of them assume the character of giant cells, immature cells in the process of division.

The coexistence of spongioblastic tumors with lesions of tuberous sclerosis has been noted previously and regarded as an expression of a disseminated blastomatous process with the main tumor mass being a local accentuation of the more or less generalized neoplastic tendency (Globus [15]). The same explanation may be given for the frequency with which subependymal granulations were noted in tumors of our series. However, a distinction must be made between subependymal granulations of the neoplastic type and those usually found in subacute diffuse ependymitis (Globus and Strauss [34]) and chronic lesions such as those found in dementia paralytica. The inflammatory lesions are more likely to contain necrotic tissue zones and show adventitial infiltration by mesodermal cells. It seems close at hand to advance an explanation for the not infrequent occurrence of multiple centers of neoplastic growth as revealed in a fairly large number of our cases. This can be found in the mode of origin of these tumors from disseminated embryonal vestiges in the widespread subependymal cell plate.

Another noteworthy observation is that the spongioneuroblastoma can be recognized grossly by the peculiar rubbery feel and the smooth, gelatinous, often slimy, oyster-like surface to which Schwarz, Goolker and Globus [11] have previously drawn attention.

In true spongioblastoma ependymale we were unable to find definite evidence of the presence of the cytoplasmic granules referred to as blepharoplasts. In a larger group of tumors classified as ependymoma, studied in this laboratory (Weinstein [35]), no such structures could be distinguished. It seems that they are not essential for the recognition of such tumors. The Heidenhain method, as well as silver impregnation, is capable of revealing granules of various origins, mitochondria among others, and hence is not reliable in this respect and leads to errors.

A Few Clinical Observations.—The general clinical manifestations of tumors of this series do not deviate much from those of brain tumors of other types. Signs of increased intracranial tension, such as headache, nausea, vomiting, papilledema and disturbances of vision, were found in varying degrees in all these cases. As regards focal signs, such as hemiplegia and hypalgesia, there were also no striking variations

34. Globus, J. H., and Strauss, I.: Arch. Neurol. & Psychiat. **19**:623, 1928.
35. Weinstein, E. A.: J. Mt. Sinai Hosp. **5**:573, 1938.

from the manifestations noted in tumors of other forms, wherever a direct relationship between such signs and the location of the tumor was present.

A search for some special clinical features yielded few significant findings. They will be presented in order of their greater prevalence:

Somnolence as a prominent symptom was noted in 11 cases (1, 4, 6, 7, 9, 11, 15, 18, 19, 20, 21).

Convulsive seizures, some of the jacksonian variety, were encountered in 5 cases (2, 11, 18, 19, 21).

Aphasic disturbances were observed in 5 cases (4, 5, 10, 13, 18). In case 5 the destruction of the subcortex in the region of the insula was associated with a mixed type of aphasia. In case 13 a mild motor aphasia revealed a lesion in a similar location. In case 18 the aphasic manifestations were associated with the involvement of the anterior part of the corpus callosum. In case 10 motor and sensory aphasia as a late manifestation was associated with widespread destruction of the occipital and parietal lobe. In case 4 a slight motor aphasia disclosed no known anatomic basis and probably finds its explanation in a possible functional alteration in the speech area caused by increased intracranial tension.

Emotional changes were observed in 7 cases. Although the anterior nucleus of the thalamus is considered to bear an important relation to emotional activity and this region was found to be involved in 9 cases, it is rather striking that in only 2 of these (cases 13 and 17) were emotional changes evident. Of the other 5 cases in which emotional unbalance was a feature, in 1 (case 3) there was no definite lesion in the anterior nuclei but there was extensive destruction of the neighboring thalamic nuclei; in another (case 9) the hypothalamus was invaded by a neoplastic focus; in another (case 2) the tumor involved the subcortex of the right frontal lobe, the right septum pellucidum and the corpus callosum, while in another case with multiple tumors (case 1) the only lesions which could explain the emotional changes implicated the anterior part of the corpus callosum dorsal to a neoplastic focus originating in the septum pellucidum.

Transient sensory hallucinations were observed in only 2 cases in which the tumors involved the vicinity of the sensory, or presumably sensory, areas. In one (case 19), in which transient visual hallucinations were noted, the centrum semiovale of the occipital lobe was implicated. In a case with olfactory hallucinations (case 10) the medial part of the parietal lobe near the retrosplenial area was involved. This area, according to von Economo,[36] might be related to the olfactory sense.

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36. von Economo, C.: Zellaufbau der Grosshirnrinde des Menschen, Berlin, Julius Springer, 1927.

Thalamic pain was found in 1 instance (case 13), involving almost the entire thalamus, including the ventral nuclei.

Vegetative dysfunctions, i. e., hyperthermia, obstipation and hypersomnia, were noted in case 9, with a tumor situated in the hypothalamus.

As to parkinsonian and related manifestations, in at least 10 cases of this series there was definite involvement of both components of the lentiform nucleus, the putamen and the globus pallidus, either through compression or destructive invasion. It is therefore remarkable that the features of the parkinsonian syndrome or extrapyramidal manifestations, such as rigidity, tremor, athetosis and choreiform movements, were rare or absent. Only in 3 cases were symptoms shown which could be referred to striopallidal lesions. In 1 instance with severe compression of the entire left lenticular nucleus (case 3) choreiform movements of all extremities and grimacing were reported. In another instance (case 11), with compression of the left lenticular nucleus, tremor and rigidity of the right hand were observed. In still another case with a similar anatomic involvement (case 6) shaking of the right arm was noted.

Deafness on the right side and homolateral with the tumor invasion of both dorsal and ventral cochlear nuclei in the lateral recess of the fourth ventricle was found in case 1.

The age incidence when analyzed disclosed no important data; the tumors were encountered in patients between the ages of 15 and 65 years; the great majority of the patients, however, were between the ages of 20 and 45 years.

Sex distribution has yielded some data which should be noted, but the number is too small to permit a deduction. Of the 21 patients, 8 were males and 13 females.

The duration of the clinical course in a large number of cases was very short. Thus, in 8 cases the entire length of the illness did not exceed three months; in 3 cases it did not exceed one year, and in the rest the period varied between two and eight years. Since the largest number of cases fell into the group of spongioneuroblastoma, the short course of the larger number of cases is readily understood.

The association of the spongioneuroblastoma with tuberous sclerosis is another observation which is in accord with the results of previous studies by one of us.[37] It seems to be in harmony with the concept that spongioblastic, and particularly spongioneuroblastic, tumors are in the main products of a blastomatous process giving expression to its neoplastic potencies.

SUMMARY

Evidence is presented to emphasize the significance of embryonal and histogenetic factors in the production of brain tumors.

37. Globus.[15] Globus and others.[27a]

It is demonstrated that these factors are operative particularly in the region designated as the striatothalamic junction, including the sulcus terminalis, and in other areas—the zone of coalescence of the anterior horns of the lateral ventricles and the subependymal cell plate about the nucleus caudatus and the septum pellucidum.

These zones, the striatothalamic and others, have been found to be frequent sites of tumor formation. The explanation for this is to be found in the fact that they constitute common sites for embryonal residues.

The tumors most commonly found in these situations are identified in accordance with a simple classification. The spongioneuroblastic variety is the most frequent, although others such as spongioblastoma multiforme or ependymale, as well as transitional forms, are occasionally encountered there. Tumors of the spongioneuroblastic variety are not infrequently multiple, with their distribution in zones of predilection for embryonal residues. The primitive character of embryonal residues found in the aforementioned locations is in all probability responsible for the character of the tumors developing there.

Further evidence has been presented that the spongioneuroblastoma and its transitional forms are of a type of tumor in which both neural and glial elements coexist in varying ratios.

Tuberous sclerosis has been found frequently associated with the spongioneuroblastic variety of tumor, and the fact that numerous ependymal granulations are frequently found in spongioneuroblastoma and tuberous sclerosis again points to the significance of the subependymal cell plate as the source of neoplastic alterations.

Clinically this type of tumor is characterized mainly by the rapidity of the unfolding of the clinical picture in a large number of cases, by the frequency of convulsive seizures, by the depreciation of alertness and by somnolence as a frequent manifestation.

ANATOMIC CHANGES IN THE PROSTATE OF PATIENTS WITH CIRRHOSIS OF THE LIVER

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In recent years evidence that the liver is the chief, if not the only, organ concerned in the inactivation of estrogens has been presented by a number of workers, both as regards animals [1] and as regards man.[2] The occurrence of gynecomastia in some patients with cirrhosis of the liver has been offered as an indication of estrogenic overactivity,[2] and the finding of increased excretion of estrogens in the urine of some of these patients has further strengthened this conclusion.[2] It follows that persons suffering from cirrhosis of the liver, with the postulated preponderance of estrogens, would show anatomic changes characteristic of estrogenic stimulation in the secondary sex organs, such as the prostate. It was therefore deemed profitable to undertake a morphologic study of the prostates of patients with cirrhosis of the liver in an attempt to ascertain whether or not characteristic anatomic alterations occur. Such changes, if found, would furnish further evidence that the normal liver inactivates estrogens and that the lesions of hepatic cirrhosis interfere with this function of inactivation.

MATERIAL AND METHOD

The present study is based on an investigation of all cases of cirrhosis of the liver in men observed in the department of pathology of Washington University School of Medicine from 1910 to 1941. There were 82 instances in a total of 9,315 autopsies. Of the 82, those in persons over 41 years of age—71 in number—are used for the statistical study.

Sections of the prostate, stained by the hematoxylin-eosin and Masson methods, were available in 13 cases. The ages of the patients ranged from 50 to 78 years.

A survey of the clinical records of the 82 male patients with cirrhosis of the liver at all ages has been made, to see if any additional indication of estrogenic

From the Department of Pathology, Washington University School of Medicine.

1. Zondek, B.: Lancet **2**:356, 1934. Israel, S. L.; Merenze, D. R., and Johnston, C. G.: Am. J. M. Sc. **194**:835, 1937. Golden, J. B., and Sevringhaus, E. L.: Proc. Soc. Exper. Biol. & Med. **39**:361, 1938. Talbot, N. B.: Endocrinology **25**:601, 1939. Pincus, G., and Martin, D. W.: ibid. **27**:838, 1940.

2. Edmondson, H. A.; Glass, S. J., and Soll, S. N.: Proc. Soc. Exper. Biol. & Med. **42**:97, 1939.

preponderance could be found, such as gynecomastia, changes of body contour, voice or distribution of hair or abnormalities of the external genitalia. No positive evidence has been obtained from a study of these records, all of which had been taken with no special attention to these points.

The incidence of nodular hyperplasia of the prostate in general is based on a total of 1,139 autopsy records on men 41 years of age or older (from no. 5100 to no. 9315, excluding nos. 8100 to 8315, Washington University department of pathology).

INCIDENCE OF NODULAR HYPERPLASIA ("BENIGN HYPERTROPHY")

The incidence of nodular hyperplasia of the prostate observed grossly in the routine autopsies in this laboratory is 9.1 per cent. Among 71 cases of cirrhosis of the liver there were 5 in which such hypertrophy was grossly demonstrable—an incidence of 7.1 per cent. Thus it is obvious that on the basis of the gross pathologic examination, this change in the prostate is no more frequent in patients with cirrhosis of the liver than in patients without cirrhosis.

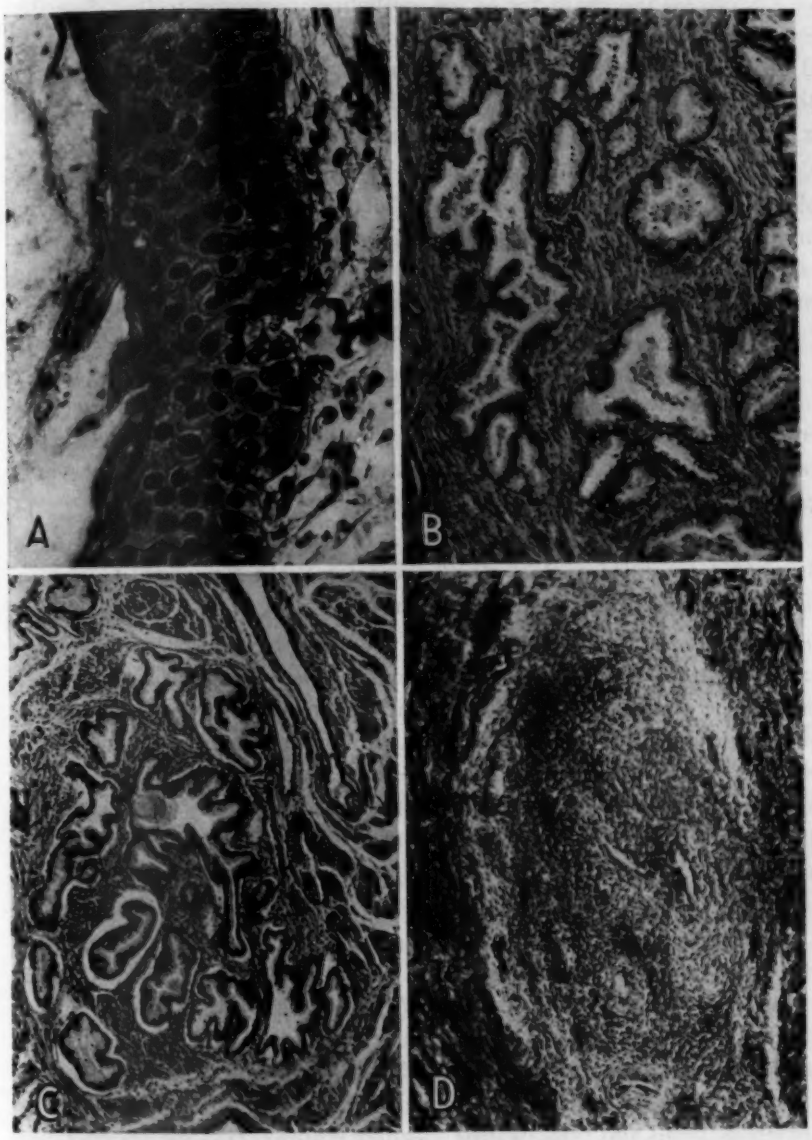
On the other hand, when the prostates are examined microscopically, something very different is revealed. According to Moore,[3] the incidence of microscopic evidence of nodular hyperplasia in 228 men between the fourth and seventh decades of life is 50.5 per cent. In the present series, if 2 cases are excluded, in which the sections of the prostates were not taken from the part of the organ where hypertrophy is usually found, all but 1 of the 11 prostates show microscopic lesions of the disease, and in the 1 exception there is suggestive evidence of the same condition. Expressed in comparable figures, the incidence is 91 per cent.

The structural changes interpreted as nodular hyperplasia, or benign hypertrophy, in the present series are the same as those used by Moore [4] and consist of both stromal and fibroglandular changes. Stromal nodules are found in all 10, and the fibroglandular type of hyperplasia is present in 6 of the 10 prostates.

MICROSCOPIC DESCRIPTION OF PROSTATES

The epithelium of the urethra, as shown in sections of the 12 prostates, consists of multilayered cells, with those along the surface showing a columnar arrangement. In one instance the section contains no urethra. Metaplasia to a squamous type of epithelium is found in 2 prostates (fig., *A*). In both of these the metaplastic epithelium shows distinct intercellular bridges and superficial keratinization. Occasional mitotic figures are present in the epithelial cells in 1 of the 2.

3. Moore, R. A.: J. Urol. **33**:224, 1935.
4. Moore, R. A.: Am. J. Path. **12**:599, 1936.



A, metaplasia of the urethral mucosa to a squamous type of epithelium; × 425. *B*, columnar secreting epithelium in glands surrounded by relatively fibrous stroma in the prostate of a man 62 years of age; × 70. *C*, fibroglandular hyperplasia with hyperplastic glands and cellular stroma; × 75. *D*, nodule made up of fibrous tissue and numerous vascular spaces; × 95.

The urethral evaginations in 5 instances show a many-layered epithelium with columnar secreting cells along the surface. In the sections of 3 prostates the character of the epithelium is similar to that described except that the surface cells show no evidence of secretory activity. In 2 instances the epithelium is multilayered but has no columnar cells on the surface, and in 1 the epithelium is simple columnar. Metaplasia to a transitional type of epithelium is found in 1 prostate.

The stroma immediately beneath the urethra in 10 of the 13 prostates shows cellular infiltration. In 6 of these the infiltration is lymphocytic, and in 1 there are definite nodules of lymphoid tissue. The infiltrating cells consist of lymphocytes, plasma cells and occasional polymorphonuclear leukocytes in 3 cases and of a mixture of lymphocytes and plasma cells in 1. No infiltration is found in 3 instances.

The periurethral tissue farther away from the urethra presents a loose and edematous appearance in 3 prostates. In 2 others there are embedded in this tissue numerous thick-walled vascular spaces, each surrounded by a zone of edematous fibrous tissue. In 1 instance the periurethral stroma consists of a dense connective tissue.

Metaplasia to the transitional type of epithelium is observed in the ducts of 4 prostates, in 1 of which there are slight superficial keratinization and edema of the surrounding stroma.

In all cases the prostatic glands are lined by an epithelium, which varies from tall columnar to low cuboidal cells. Metaplasia to a transitional type of epithelium without keratinization is observed in the glands of 1 prostate. Reduction of glands to simple acinous structures is observed in 7 instances, and complete disappearance of certain glands with replacement by hyaline fibrous tissue is found in 3. Glands containing unusually tall columnar epithelium are present in 6 of the 13 prostates (fig., *B*). In 5 of these, the glands are found in circumscribed areas as definite fibroglandular nodules (fig., *C*).

In 10 instances there is focal hyperplasia in the stroma of the prostate proper, where small fibrous nodules, sharply demarcated from the surrounding tissue, are observed (fig., *D*). A relative increase of fibrous tissue, spread diffusely throughout the stroma is found in 5 prostates. In 2 instances both types of stromal change are present. In 5 of the prostates showing focal stromal hyperplasia, the fibrous nodules contain numerous small and thick-walled vascular spaces. Hyaline degeneration of the smooth muscle fibers of the stroma is observed in 4 instances: in 2 this change is extensive, in 1 moderate and in the remaining 1 only occasional fibers are involved. Lymphocytic infiltration of the stroma is found in 8 instances.

The ejaculatory canal is shown in the sections of 2 prostates. In 1 it is found to be atrophic, and in the other the muscle tissue in it is prominent and richly cellular. The utricle is not shown in any of the sections.

In the sections of 6 prostates there are many corpora amylacea, in 2 there are a few, and in 5 there are none. The sections of 2 prostates are taken from the part of the gland not usually involved in benign hypertrophy. Of the other 11, in which it is possible to study the periurethral tissue and the lateral lobes, definite evidence of benign hypertrophy is found in 10, and suggestive evidence is present in the remaining 1.

COMMENT

Progress of Senile Involution.—In 8 of the 13 prostates examined microscopically, the structural appearance indicates an age less than the

Comparison of Estimated and Actual Age of Prostate and Its Significance

Autopsy Number	Estimated Age, Yr.	Actual Age, Yr.	Difference	Interpretation
9082	48	50	− 2	
5330	63	53	+10	Advanced senility
7228	58	54	+ 4	
6953	60	55	+ 5	
6961	58	55	+ 3	
8821	58	56	+ 2	
6352	47	57	−10	Delayed involution
6477	50	62	−12	Delayed involution
8529	52	63	−11	Delayed involution
8382	54	65	−11	Delayed involution
8976	64	69	− 5	
9095	49	73	−24	Delayed involution
6601	53	78	−25	Delayed involution
Average	54.9	60.8		

actual age. In only 1 of the remaining 5 is the age estimated as much as ten years more than the chronologic age.

The incidence of delayed senility or involution in the general group of autopsies as determined by Moore [4] is different from the incidence given here in that there is a high percentage of such cases in the fourth and fifth decades, falling off markedly in the sixth decade, and disappearing in the later ones. It therefore appears that the present group of prostates shows a tendency toward delayed senility in a direction the reverse of that ordinarily found, suggesting abnormal preservation of the presenile structures of the prostates during the seventh and eighth decades.

The same conclusion is brought out by comparing the averages of the estimated and the actual ages in the present series with the averages determined by Moore,[4] who obtained a close agreement. Obviously, something is at work which is preventing the structures of the prostate

from undergoing involution. Since the removal of testes in animals brings about prostatic atrophy, with the same general type of change as in senile involution in man, and since the restoration of these structures to normal can be accomplished by the administration of testicular or male urinary extracts, the interpretation of the aforementioned finding is obvious. The prostate of the patient with cirrhosis of the liver is stimulated by larger amounts of the hormones responsible for the maintenance of the normal structure and hence does not undergo involution. It is not possible to state what hormones are active in this physiologic process, androgens, estrogens or both. The studies of Biskind and Mark [5] and of Glass and others,[6] demonstrating that the liver inactivates androgens as well as estrogens, make probable an activity of either or both in persons with cirrhosis.

Nodular Hyperplasia.—A comparison of the incidence of nodular hyperplasia in the present group of cases with that in an ordinary group reveals a conspicuous difference. In an average group there is a gradual and steady increase of the incidence with advancing age with an average of 50.5 per cent in persons 41 years old or more; in patients with cirrhosis the percentage in the lower age groups is high instead of low. It is particularly striking that in this series the highest incidence of hyperplasia is in the sixth decade, a decade during which the general incidence is still quite low. The present series leaves much to be desired in number of observations, but the consistent results in an unselected series support the conclusion that histologic evidence of nodular hyperplasia is more common in patients with cirrhosis of the liver.

It is to be noted that none of the prostates shows any gross evidence of enlargement, and that the microscopic changes are only the beginnings of nodular hyperplasia. Had the patients lived longer, the nodules might have continued to grow to sufficient size to become clinically manifest. The fact that the patient with cirrhosis of the liver has nodular hyperplasia of the prostate and a preponderance of estrogens lends further support to the theory that the etiology of benign prostatic hypertrophy is in part the result of an estrogenic preponderance.

Epithelial Metaplasia.—The metaplasia of the epithelium in the urethra to a squamous type with keratinization in 2 patients, 55 and 62 years old, and the keratinization of a transitional type of epithelium in the ducts of the prostate of a man 54 years old are further indications of exaggerated estrogenic effect.

Possible Hormonal Explanation of the Foregoing Findings.—The preservation of certain structures in the prostates of men with cirrhosis

5. Biskind, G. R., and Mark, J.: Bull. Johns Hopkins Hosp. **65**:212, 1939.

6. Glass, S. J.; Edmondson, H. A., and Soll, S. N.: Endocrinology **27**:749, 1940.

of the liver, in whom such structures should normally have undergone involutionary changes; the occurrence of microscopic evidence of nodular hyperplasia in almost every case in the series, and the finding of epithelial metaplasia with keratinization in some of them point to estrogenic preponderance. Since the histologic changes in the breasts of the patients with cirrhosis of the liver who showed gynecomastia have been claimed to be comparable to those induced by injections of estrogens, it is reasonable to believe that the same agent which causes the gynecomastia is responsible for the changes in the prostates. The question immediately suggests itself: What is the source of the increase in estrogen? In view of the evidence presented in the literature that the normal liver inactivates estrogens, it is held that when the liver is diseased, as in cirrhosis, it fails to perform that function and allows more hormone to exert its influence on the secondary sex organs.

SUMMARY

From the microscopic observations on 13 prostates from patients with cirrhosis of the liver, the following conclusions are drawn: The prostates of patients with cirrhosis of the liver in and beyond the fifth decade of life show a greater incidence of nodular hyperplasia (benign hypertrophy), particularly in the younger age groups, than is found in routine autopsies on men over 40.

These prostates also show a higher incidence of delayed involution, especially in the older age groups of the seventh decade. The occurrence of epithelial metaplasia with keratinization in a few of the prostates studied is greater than can be accounted for by involutionary changes caused by age.

All the foregoing findings are interpreted as evidence of an estrogenic preponderance occurring in patients with cirrhosis of the liver, and indirectly they lend support to the belief that one of the functions of the normal liver is the inactivation of the sex hormones, particularly of the estrogens. The high incidence of microscopic evidence of nodular hyperplasia gives indirect support to the theory that this disease is caused by, or at least is related to, estrogenic preponderance.

Case Reports

OXYURIS VERMICULARIS INFECTION OF THE WALL OF A FALLOPIAN TUBE

B. Chomet, M.D., Elyria, Ohio

Dec. 23, 1938, a white woman aged 32 years was admitted to the hospital in shock after having been shot in the buttock, wall of the chest, right flank and abdomen. Roentgen examination showed opaque foreign bodies in the region of the muscles of the back, apparently posterior to the stomach. The patient recovered in about six weeks without operation and without clear indication of peritonitis. July 13, 1940,

Ova of Oxyuris vermicularis in fallopian tube; × 400.

she was readmitted, complaining of headache, backache and a feeling of pressure in the lower part of the abdomen. The examination showed what seemed to be a mass in the left lower quadrant. The blood count indicated slight anemia. The differential count was normal with the possible exception of the presence of 2 per cent eosinophils. At operation the right fallopian tube and ovary were removed because of marked adhesions. The anatomic diagnosis was chronic and acute salpingitis and perioophoritis with follicular cysts on the right. After fourteen days the patient was discharged well. December 21 she was admitted again, complaining of continuous backache, pain in the left side of the pelvis and dysuria, which had continued since the operation. There was enlargement of the left

From the Laboratories of the Elyria Memorial Hospital, Elyria, Ohio, and the Institute of Pathology of Western Reserve University, Cleveland.

adnexa with some tenderness. At this time the blood count was essentially normal, and no eosinophils were discovered. At operation, the left ovary and fallopian tube were found bound down by dense fibrous adhesions, and these organs were removed.

The tube was found to measure in general 7 by 1 cm. The fimbriated end was obliterated, and in this region the tube was firm and fibrotic. Near the end there were a few small yellowish white firm nodules, measuring about 6 mm. in diameter. The remainder of the serosa was irregularly thickened. Cross section showed hyperemic and swollen mucosa. Microscopic examination of the tube showed marked thickening of the plicae, lined by a single layer of high cuboidal epithelium with cilia. The supporting connective tissue was increased and showed mucoid degeneration together with moderately dense infiltration by eosinophilic leukocytes and lymphocytes. The outer layer of the muscle and the serosa were markedly fibrotic. Sections of two of the nodules showed that they were made up of an extensive pink-staining granular necrotic tissue, which contained many acicular spaces. The periphery of the nodule showed a thin layer of somewhat vacuolated fibrous tissue with moderate numbers of fibroblasts, lymphocytes, plasma cells and eosinophils. Within this layer there were a few giant cells, some of which were definitely of the foreign body type, while others closely resembled the Langhans type of giant cell but were without radially disposed peripherally situated nuclei. The peripheries of the nodules were immediately under the mucosal layer of the tube. The centers of the nodules showed numerous typical ova of Oxyuris vermicularis with well defined cuticle.

COMMENT

Froriep [1] distinguished two ways in which O. vermicularis may migrate into a fallopian tube: (1) by the genital canal and (2) by a perforation of the intestine or the appendix. He mentioned only 1 case (Bodechtel's) in which infection of the omentum may have occurred as a result of perforation of the appendix. Bodechtel resected a retroflected uterus with adherent omentum. There was an encapsulated oxyurid in the omentum. In serial sections of the appendix an area of remote perforation was found. According to Bodechtel there was a symptomless perforation with emigration of oxyurids.

All other authors [2] have agreed that the migration occurs through the genital canal. This opinion is supported by the finding of oxyurids in the vagina (Heller), in the cervix (Simons [3]) and within the tubal lumen (Tschamer [3]) and by the observation that the lesions are usually in the neighborhood of the abdominal lumen of the fallopian tube.

The supposition that oxyurids in this case entered the fallopian tube from the intestinal canal must be predicated on the view that the intestine was perforated at the time the patient was shot. The absence of the clinical features of acute peritonitis strongly indicates that the

1. Froriep, E.: Zentralbl. f. Gynäk. **62**:923, 1938.

2. (*a*) Chiari, H.: Virchows Arch. f. path. Anat. **269**:730, 1928. (*b*) Smith, W. S., and Denton, J. T.: Am. J. Obst. & Gynec. **16**:205, 1928. (*c*) Jones, W. T., and Bunting, C. H.: Arch. Path. **11**:229, 1931. (*d*) Wu, L. C.: Chinese M. J. **49**:256, 1935.

3. Cited by Chiari.[2a]

intestine was not perforated. There was no evidence at operation that the appendix was diseased. As indicated by reports of other authors, the situation of the masses of ova near the fimbriated end is consistent with transmission from the lower genital canal. Furthermore, the histologic demonstration of pseudotubercles corresponds with that of others. The assumption seems justified in this case that the presence of the ova in the tube was due to infection of the vagina and uterus.

SUMMARY

A case is reported of infection of a fallopian tube by Oxyuris vermicularis, probably due to transport from the lower genital canal.

SYPHILIS OF THE AORTA AND CORONARY ARTERIES

GEORGE STRASSMANN, M.D., AND PHILIP GOLDSTEIN, M.D., NEW YORK

Syphilis of the coronary arteries is rare. Saphir [1] examined 130 cases of syphilitic aortitis microscopically and did not find any involvement of the coronary arteries. Martland [2] from a wide experience with syphilitic lesions of the aorta stated any extension of the process beyond the coronary orifices is unusual and if present is insignificant. Moritz [3] reported 6 cases of syphilitic aortitis in which the proximal portions of the coronary arteries were involved for a distance of 12 mm. Some observers [4] have described localized syphilitic lesions of the coronary arteries occurring at different points in the course of these vessels. In all the cases the process consisted for the most part of cellular infiltration of the adventitia, obliterating arteritis of the vasa vasorum, fibrous scarring and hyalinization of the media, and marked subintimal proliferation of fibrous tissue sufficient to cause narrowing of the lumen of the main arterial trunk.

Severe syphilitic involvement of the coronary arteries throughout a larger portion of the length of the vessels with special involvement of the media is admittedly rare. We now report such a case, which was encountered among the routine necropsies of the Office of the Chief Medical Examiner of the City of New York.

REPORT OF A CASE

A white woman 35 years of age, while at a subway station, became suddenly ill with severe pain in the hypogastrium. On the way to a hospital she vomited, lost consciousness and died. No further history was available at the time of autopsy. Subsequently it was learned from her fiancé that for the past three months she had complained of pain in the chest and arms, for which she had been treated by a physician.

A necropsy was made thirteen hours after death. The principal findings were syphilitic aortitis with marked narrowing of the mouths of both coronary arteries. The orifice of the left coronary artery was normally placed on the aorta and was narrowed to a pinpoint. The right orifice was situated above the sinus of Valsalva and was narrowed to a diameter of 1 mm. The heart was not hypertrophied, and the myocardium did not reveal any scars. The valves did not show evidence of inflammation. There was marked concentric thickening of the branches of the

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From the Office of the Chief Medical Examiner of the City of New York and the Department of Forensic Medicine of New York University.

1. Saphir, O.: Arch. Path. **13**:266, 1932; Am. J. Path. **5**:397, 1929. Saphir, O., and Scott, R. W.: Am. Heart J. **6**:56, 1930.

2. Martland, H. S.: Am. Heart J. **6**:1, 1930; Bull. New York Acad. Med. **8**:451, 1932.

3. Moritz, A. R.: Arch. Path. **11**:44, 1931.

4. Warthin, A. S.: Am. Heart J. **1**:1, 1925; Am. J. Syph. **2**:425, 1918. Brooks, H.: ibid. **5**:217, 1931. Maher, C. C.: Am. Heart J. **6**:37, 1930. Norris, J.: J. A. M. A. **108**:168, 1937.

coronary arteries throughout their course. On section the thickened arteries were grayish. The yellow color of atheromatous degeneration, the eccentric thickening of the wall and the calcification usually found in arteriosclerosis were not present.

Microscopically, in the aorta above the valve cusps there were intense infiltration of the aortic adventitia and media by lymphocytes and plasma cells, destruction of the elastic fibers with scar tissue formation, intimal proliferation and obliterating endarteritis of the vasa vasorum.

Striking changes were found in the anterior descending branch of the left coronary artery. In sections through the artery 2 cm. from the mouth (figs. 1

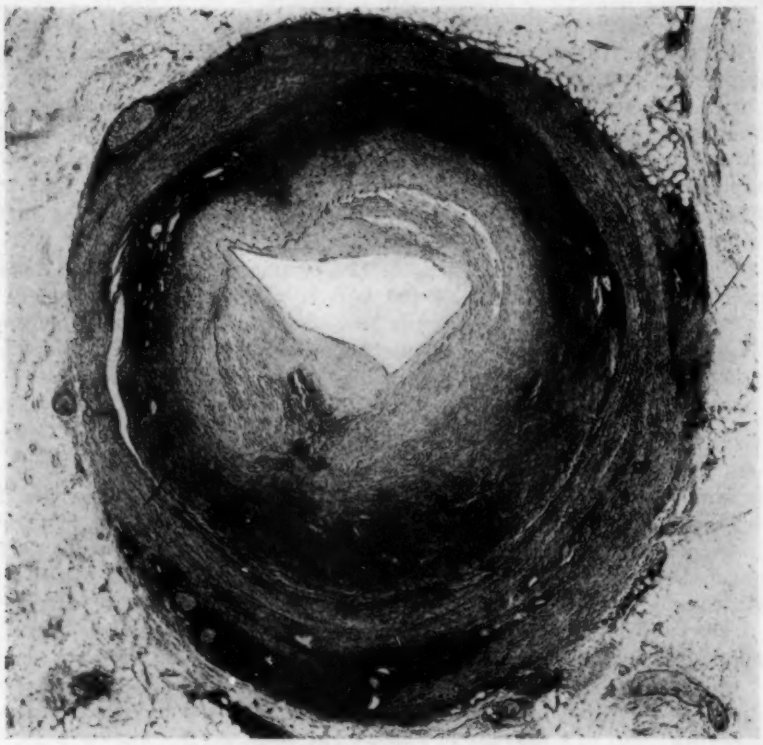

Fig. 1.—Cross section of the descending branch of the left coronary artery, showing dense cellular infiltration of the adventitia and the media, many newly formed blood vessels in the media and intimal proliferation resulting in narrowing of the lumen. Hematoxylin-eosin stain; Summar objective; × 15.

and 2) slight periadventitial and intense adventitial infiltration by lymphocytes and plasma cells was seen. There was also obliterating endarteritis of the vasa vasorum of the adventitia. The most pronounced lesion was found in the media of the left coronary artery, which was completely encircled by a dense infiltrate of lymphocytes and plasma cells. In a section stained for elastic tissue this cellular infiltrate was seen to extend from the adventitia in some places as far as the intima. There were newly formed blood vessels in the inflamed area; the elastic fibers had disappeared

in the zone of inflammation. In addition there were destruction of elastic fibers, necrosis and scar tissue formation in other areas of the media. The intima showed an area of marked fibroblastic proliferation with narrowing of the lumen of the vessel. Spirochetes could not be demonstrated in sections treated with Levaditi stain.

Similar perivascular infiltration of the adventitia, obliterating endarteritis of the vasa vasorum and intimal proliferation were seen in sections of the left circumflex

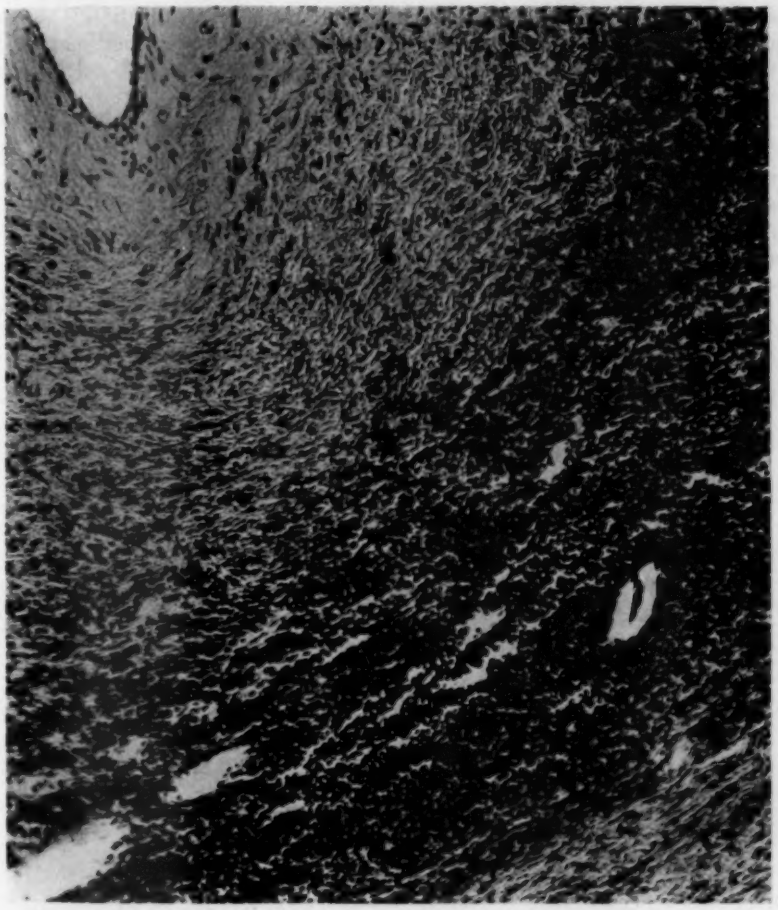

Fig. 2.—Higher magnification of a portion of the section seen in figure 1, showing dense cellular infiltration of the media with newly formed blood vessels, some of them with obliterating endarteritis, and marked intimal proliferation; 16 mm. objective.

branch and of the right coronary artery. The media in these sections did not show any changes except atrophy.

These microscopic changes represent syphilitic arteritis with special involvement of the media of the anterior descending branch of the left coronary artery.

COMMENT

Sudden death caused by syphilitic aortitis associated with stenosis or occlusion of the orifice of one or both coronary arteries is fairly common.[5] Syphilitic aortitis is often associated with arteriosclerosis of the coronary arteries.[6] Seventeen of 28 cases of sudden death caused by syphilitic aortitis with stenosis of the mouths of the coronary arteries investigated by the Office of the Chief Medical Examiner of the City of New York in Manhattan during the years 1940 and 1941 showed moderate or advanced coronary arteriosclerosis. A combination of syphilitic aortitis with syphilis of the coronary arteries distal to their orifices is, however, rare.[7]

The case which we report showed a combination of syphilitic aortitis and syphilitic coronary arteritis. The aortitis produced stenosis of the orifices of both coronary arteries and the arteritis resulted in marked narrowing of the lumen of the main branches of both vessels. In combination, these two processes impaired the blood supply of the myocardium and were undoubtedly responsible for the sudden death.

5. Bruenn, H. G.: Am. Heart J. **9**:421, 1934. Pincoffs, M. C., and Love, W. C.: Am. J. Syph. & Neurol. **18**:148, 1934. Martland.[2] Saphir.[1]

6. Saphir.[1] Martland.[2]

7. Martland.[2] Saphir.[1]

General Reviews

EFFECTS OF RADIATION ON NORMAL TISSUES

SHIELDS WARREN, M.D.
BOSTON

(*Continued from Page 608*)

IV. EFFECTS OF RADIATION ON THE GASTROINTESTINAL TRACT, INCLUDING THE SALIVARY GLANDS, THE LIVER AND THE PANCREAS

NATHAN B. FRIEDMAN, M.D.

THE STOMACH AND THE INTESTINE: EXPERIMENTAL STUDIES

Experimental observations on the effect of radiation on the gastrointestinal tract have emphasized mainly the striking damage suffered by the intestinal mucosa, leading to extensive ulceration, bloody diarrhea, marked prostration and often to death.[1] The constitutional reaction following irradiation of the stomach alone is less severe.

A great deal has been written about the "relative sensitivity" of the stomach and the intestine to radiation. On the one hand, several workers [2] have stated that the intestine is twice as sensitive as the stomach. When both structures receive approximately the same amount of radiation, the intestinal damage either may be unaccompanied by changes in the stomach [3] or may be more profound than the changes in the stomach. On the other hand, definite degenerative changes in some of the epithelial cells of the gastric mucosa have been seen following extremely low doses (Engelstad, 1935, 1938).

Some investigators have expressed the belief [4] that the small intestine is more sensitive than the large intestine. Most of the reports deal with the effect of single doses and are concerned mainly with early acute degenerative changes. However, in one study (Warren and Friedman) in which repeated doses were given, chronic ulcerating lesions were found predominantly in the large intestine.

From the laboratories of pathology of the Harvard Cancer Commission, New England Deaconess Hospital and Pondville State Hospital for Cancer, and the department of pathology of Harvard Medical School.

1. Regaud and others. Hall and Whipple. Moon and others.
2. Regaud and others. Ivy and others (1923, 1924). Schürch and Uehlinger.
3. Mottram. Hall and Whipple. Warren and Whipple (1922 a).
4. Warren and Whipple (1922 a). Tsuzuki. Lazarus-Barlow.

Engelstad's data (1935, 1938) showed a decided difference in the effects of radiation given in a single massive dose and the same total quantity given in divided doses. Fractionation produced fewer ulcers, and the mortality was lower. Warren and Whipple (1923 e) found that maintenance of a six day interval between doses resulted in increased tolerance but that division into daily doses had no such effect.

The particular radiation effect used in estimating sensitivity has not been constant. It is not clear whether "sensitivity" should be indicated by the threshold of damage or by the relative damage sustained at higher doses.

Difficulties are apparent enough when one considers the effect of radiation on the different portions of the gastrointestinal tract, which under most conditions receive comparable "depth doses," and these difficulties are enhanced when one considers the "relative sensitivity" of the skin in relation to that of the stomach and the intestine. Depth doses are subject to much controversy as well as much error.

Species differences should be mentioned at this point. Cutaneous erythema doses vary considerably in different animals, although the so-called "intestinal doses" may be more comparable. Nonmammalian forms, such as the frog, have tolerated doses from two to twenty times those lethal for mammals (Warren and Whipple, 1923 g; Lazarus-Barlow).

For these reasons the formulation of any general statement regarding "sensitivity" is being evaded. The data, however, do indicate that morphologic changes of comparable degree and character take place in both the stomach and the intestine after doses of radiation that are of approximately the same order of magnitude.

Methods.—The procedure employed by most workers has been to expose the whole abdomen or a portion of it to radiation, using single or multiple fields. Usually roentgen rays or the gamma rays of radium were employed, but neutrons were used by Lawrence and Tennant. Although in the majority of experiments the radiation was administered in a single exposure, courses of divided doses have also been given. In some experiments radium was placed within the abdomen, in or near the organ to be studied, while in other experiments the stomach or the intestine was removed from the abdomen during the period of exposure to radiation.

The accompanying table, which gives technical details of the work of various authors, is limited to some of the more complete experiments and to those which were concerned in large part with the pathologic alterations in the stomach and intestine. It should be emphasized that it is presented mainly to give a rough idea of the order of magnitude of the doses used. In many instances the authors used several methods employing various factors. For the purpose of brevity all of these have not been included in the table, and in some cases averages or approximations are given. For exact and complete data the original reports should be consulted.

Besides those cited in the table, many other workers have studied the pathologic alterations in the gastrointestinal tract; among them are: Krause and

Ziegler (1906); Werner (1907); Fromme (1917); Keller (1921); Szego and Rother (1921); Miescher (1923); Strauss (1923); Harbinson (1924); ten Doornkaat Koolman (1932); Lawrence and Tennant (1937); Hayashi and Maruyama (1938).

Pathologic Effects

There is striking uniformity in the effect of radiation on the structure of the stomach and the intestine in the various species that have been studied, despite the marked differences in the methods employed.

The most striking changes occur in the epithelial cells and the tissue structures made up of those cells. These changes are not confined to the epithelial elements but include reactions in and of the connective tissue stroma, blood vessels, inflammatory cells, lymphoid structures, muscles, nerve tissue and serosa. Severe damage leads to destruction of the normal lining and to consequent ulceration; less severe damage leads to alteration of the structure of the mucosa without ulceration.

A minor early reaction, chiefly edema, takes place within the first days and even the first few hours after radiation. The major effect, consisting in degenerative changes in the epithelial cells of the mucosa and in the stromal structures, with hyperemia, hemorrhage and leukocytic infiltration, begins in about a week. These degenerative changes progress to ulceration, the peak of this process being reached in one to two months. After this, reparative efforts involving both epithelial and connective tissue repair are more in evidence.

Epithelial Cells.—(*a*) Cell degeneration: The most prominent features of the early reaction in the first few hours and days after irradiation of epithelial tissues are marked edema (Engelstad, 1935, 1938) with swelling of the cell nuclei and prominence of the nucleoli and chromatin.[5] The major reactions are well established as early as four days after exposure to radiation. Edema, swelling and vacuolation of both cytoplasm and nuclei, or disintegration of cells with nuclear pyknosis or karyorrhexis, have all been described. Frank necrosis and cytolysis have been observed and have sometimes been described as liquefaction necrosis or coagulation necrosis. Less badly damaged cells show shrinkage and atrophy, especially in the later stages.

(*b*) Mucinous change: Increased secretion of mucus, the so-called "mucous degeneration", is a striking feature of radiation reaction in the gastrointestinal mucosa. It is usually seen[6] when the damage is sublethal, as after small doses or in cells surviving large doses. Warren and Whipple (1922 b) and Lazarus-Barlow observed such secretion in the colon with doses that caused desquamation in the small intestine.

5. Lazarus-Barlow. Schürch and Uehlinger. Terplan. Warren and Friedman.

6. Mottram. Delbet and others. Martin and Rogers (1923). Terplan. Ivy and others (1924). Dawson (1927).

Details of Radiation Technics Used by Various Investigators

Authors	Animal	Technic
Delbet and co-workers.....	Dog	Radium (intragastric), 50 mg. for 24 hours—1,200 mg. hours
Regaud and co-workers.....	Dog	Roentgen rays. Filter, 1-4 mm. aluminum. Distance, 17-22 cm. Multiple exposures totaled 1 to 6 hours
Ghilarducci.....	Rabbit	Roentgen rays (spark gap, 30 cm.) 3 m. a. Filter, 1-4 mm. aluminum. Exposure 5 to 20 min. Some animals received barium
Hall and Whipple.....	Dog	Roentgen rays. Spark gap, 6-9 in. (15 to 23 cm.). Filter, 0-2 mm. aluminum. Lethal dose, 200 m. a. min. (with 9 in. gap). Lethal dose, 450 m. a. min. (with 6 in. gap)
Lazarus-Barlow.....	Frog Rat Rabbit Cat	Radium 5 Gm. Distance, 4-15 cm. Exposure, 3 hours to 4 days. Lethal doses: rat, 6 hours at 4 cm.; rabbit, 48 hours at 15 cm.; cat, 24 hours at 15 cm.; frog, much higher
Warren and Whipple..... (1922-1923, a to g)	Dog Cat Rabbit Guinea pig Rat Bird Frog Snake	Roentgen rays, 95 kv., 8 m. a. Filter, 2 mm. aluminum. Distance, 25 cm. Field, 6½ in. (16.5 cm.) in diameter. Doses, to 480 m. a. min. Lethal dose (dog, cat, guinea pig), about 350 m. a. min. Erythema and "intestinal" dose: dog, 100 m. a. min.; guinea pig, 200 m. a. min.; cat, 300 m. a. min.
Mottram.....	Rat	Radium, 220 mg. Filter, 0.1 mm. lead, 0.12 mm. silver. Distance, 8 in. (20 cm.). Exposure, 1 to 6 days
Martin and Rogers..... (1923-1924)	Dog	Roentgen rays to exteriorized loop, 75 kv. (spark gap 5 in. [12.5 cm.]), 10 m. a., unfiltered. Distance, 10 in. (25 cm.). Field, 3½ in. (9 cm.) square. Dose, 25 to 150 m. a. min.
.....	Guinea pig	Roentgen rays. Spark gap, 9 in. Filter, 2 mm. aluminum. Distance, 10 in. Dose, 56 to 200 m. a. min. Lethal dose, 200 m. a. min.
Ivy and co-workers..... (1923-1924) Dawson..... (1925-1927)	Dog	Roentgen rays, 100 kv., 5 m. a. Filter, 1 mm. aluminum. Distance, 30 cm. Field, 6 cm. diameter. Exposure, 30 to 60 min. Dog erythema dose, about 35 min. Human erythema dose, about 15 min. Also Roentgen rays, 200 kv., 5 m. a. Filter, 1 mm. aluminum, 0.75 mm. copper. Distance, 50 cm. Field 11 x 21 cm. Exposure, 90 to 120 min. ¾ to 1 human erythema dose
Tsukamoto.....	Rabbit	Roentgen rays, 180 kv. (spark gap, 39-45 cm.), 2-4 m. a. Filter, 0.5 mm. copper, 1 mm. aluminum. Distance, 23-30 cm. Field, 6 x 8 cm. Multiple exposures totaled to 6 hours
Cori.....	Mouse	Roentgen rays, 95 kv., 4 m. a. Filter, 2.5 mm. aluminum. Distance, 20 cm. Field, 1 x 1.5 cm. Exposure, to 80 min. Mouse erythema dose, 60 min. "Intestinal" and lethal doses, about ⅓ to ½ of erythema dose. Human erythema dose, about ¼ of mouse dose
Kolodny.....	Rabbit	Roentgen rays, 200 kg., 5 m. a. Filter, 0.5 mm. copper. Distance, 70 cm. Exposure, 1 to 2 hours
Podesta.....	Dog	Roentgen rays. Spark gap, 34 cm., 3 m. a. Filter, 2 mm. aluminum, 0.2 mm. brass. Distance, 30 cm. Field, 8 x 6 cm. Exposure, 70-420 min. (some multiple portals)
Broeq and Oury.....	Dog Guinea pig	Roentgen rays. Several exposures totaling 2,000-7,000 "Solomon ionometre R units"
Tsuzuki.....	Rabbit	Roentgen rays, 170 kv. (spark gap, 25 cm.), 2 m. a. Filter, 0.5 mm. copper, 1 mm. aluminum. Distance, 40 cm. Exposure, 15 to 240 min. Erythema dose, 150 min. Depth dose to intestine, 50 per cent. "Intestinal" dose, 60 min.
Wolfer.....	Dog	Roentgen rays to exteriorized gastric mucosa, 110 kv. (spark gap, 6 in.), 5 m. a., unfiltered. Distance, 9 in. Field, 3 in. (7.5 cm.) in diameter. Exposure, 30 min.

Details of Radiation Technics Used by Various Investigators—Continued

Authors	Animal	Technic
Aue and Ceculin	Dog	Radium by local implantation, 3-7 mc. Maximal dose, about 900 mc. hours
Buchwald	Rat	Roentgen rays, 140 kv., 5 m. a. Filter, 2.5 mm. aluminum. Distance, 25 cm. Exposure, 10 min. Lethal dose, 13 min.
Snell and Bollmann	Dog	Roentgen rays, 130 kv., 5 m. a. Filter, 3 mm. aluminum. Exposure, 22 to 114 min. Maximal dose, 13,000 r
Chrom	Mouse	Roentgen rays, 160 kv., 4 m. a. Filter, 3 mm. aluminum, 0.5 mm. copper. Distance, 42 cm. Field, 1 x 1.5 cm. and whole animal. Doses, 550 to 1,100 r. 80 per cent mortality with 550 r to whole animal
Schürch and Uehlinger	Rabbit Dog	Radium by local implantation, 13.3 mg., 24 to 48 hours. Maximal dose, 600 mg. hours
Engelstad (1935-1938)	Rabbit	Roentgen rays, 175 kv., 4 m. a. Filter, 0.5 mm. copper, 1 mm. aluminum. Distance, 27 cm. Field, 4 x 6 cm. Single and multiple exposures with maximal dose, 4,500 r. Transthoracic doses with maximal dose, 15,000 r. Rabbit erythema dose, 1,700 r. Intestinal ulceration with 2,000 r. High mortality with 3,000 r

Increased secretion of mucus, with viscid masses filling the intestinal lumen, may be associated with distention of the goblet cells with secretion or even with the presence of more mucus-containing cells than are normal in a given region.

On the other hand, still higher doses have caused the disappearance of goblet cells (Ivy and co-workers, 1924). Cells occasionally show "vacuolar degeneration" (Terplan; Fromme). Sometimes the vacuoles contain material which resembles mucus but stains somewhat differently. Mottram (1923) labeled this phenomenon "pseudo-mucinous degeneration" and observed that it was associated with cessation of the secretion of mucus. Mottram also described degenerating cells containing hyaline droplets.

(*c*) Mitosis: Mitotic figures, fairly abundant in control material, are diminished or absent from the intestinal crypts in the first hours after exposure to radiation.[7] They return, however, a few days or a week after cessation of the treatment. In animals irradiated every second day the mitotic figures disappear at first, but they reappear despite continued application of radiation (Warren and Friedman). Some observers have expressed the belief that the mitotic figures which reappear are more numerous than normal (Chrom); others consider that deformed figures are present (Lazarus-Barlow); or that there is an actual chromosmal increase (Dawson, 1927). Dustin described striking cellular inclusions, staining with iron-hematoxylin, which appeared with the cessation of mitotic activity and disappeared with its resumption.

(*d*) Atypical cell formation: The appearance of abnormal and unusual cell types in the mucosa after exposure to radiation has attracted considerable attention. Those most commonly seen are "undifferen-

7. Lazarus-Barlow. Terplan. Warren and Friedman.

tiated" epithelial cells,[8] which are flat to cuboidal in shape and usually basophilic. They sometimes show mitotic figures and frequently are irregular in size and shape and in nuclear structure. Multinucleate giant cells and syncytia have repeatedly been observed.[9] These changes occur particularly in later periods following irradiation of the tissues, especially at the edges of ulcers or where there has been destruction of the epithelial surface. They are found as well, however, in the remnants of glands lying within damaged mucosa. For these reasons many workers have considered them to be regenerative changes.

Nonepithelial Elements.—(*a*) Connective tissue: The connective tissues of the mucosa and submucosa, as well as the intermuscular and subserosal stromal elements, show marked changes both in the absence and in the presence of ulceration. Edema and swelling, and gelatinous, myxomatous or hyaline alteration, with loss of the normal fibrillary appearance of collagen, take place.[10] So-called fibroblast "paralysis," or fixed cell hypertrophy, is revealed by the presence of giant nuclei and bizarre cytoplasmic forms without proliferation or mitosis and without the formation of collagen.[11]

Inhibition of connective tissue proliferation may persist for two months after exposure to radiation (Engelstad, 1935, 1938). Considerable fibrosis may take place in the later stages (Regaud and co-workers). However, Brocq and Oury found little fibrosis in guinea pigs (scurvy?).

(*b*) Blood vessels: Most of the studies of vascular changes disclose relatively minor alterations. Hyperemia, thrombosis and hemorrhage occur especially in acute processes,[12] commonly in association with ulcerating lesions. The hemorrhages which occur after intravenous injections of preparations of thorium (Desjardins) are not always limited to the intestine, and they may be secondary to changes in the blood elements, although their predilection for the colon has been attributed to a local effect. Furthermore, thorium may exert a local toxic effect apart from its rather limited radioactivity.

Swelling of the endothelium with bulging into the lumen and desquamation are described,[13] most marked in the veins and least marked in the arterioles (Lazarus-Barlow). Endophlebitis was seen in 1 animal

8. Szego and Rother. Tsukamoto. Chrom. Ivy and others (1924). Dawson (1925; 1927).

9. Schürch and Uehlinger. Delbet and others. Brocq and Oury. Tsukamoto. Engelstad (1938). Fromme. Dawson (1927).

10. Delbet and others. Lazarus-Barlow. Schürch and Uehlinger. Warren and Friedman. Martin and Rogers (1923).

11. Ivy and others (1924). Dawson (1927). Delbet and others. Maximow.

12. Ivy and others (1924). Dawson (1925). Warren and Whipple (1922 a). Warren and Whipple (1922 b). Szego and Rother. Ghilarducci. Delbet and others. Kolodny.

13. Dawson (1927). Ivy and others (1924). Kolodny.

by Schürch and Uehlinger. They, as well as others (Martin and Rogers, 1924), however, commented on the paucity of obliterative vascular changes despite ulceration.

Hyaline thickening, degeneration and vacuolation of vessel walls are seen in the later and more chronic lesions.[14] Telangiectasia is common and includes the lymphatics (Warren and Friedman).

(*c*) Inflammatory cells: Frequently there is infiltration of damaged and ulcerated mucosae by inflammatory cells of mixed type.[15] Such infiltration is sometimes marked in the subepithelial and submucosal tissues although there is no ulceration, and sometimes it is scanty despite marked ulceration.

Extensive polymorphonuclear leukocytic infiltration was seen by some workers,[16] while others described predominance of lymphocytes (Kolodny) or macrophages (Engelstad, 1935, 1938) in some instances. Occasionally the absence of polymorphonuclear leukocytes, possibly due to radiation leukopenia, was noted.[17] In Engelstad's series (1935, 1938) the early infiltration by polymorphonuclear leukocytes was frequently replaced at a later stage by lymphocytic infiltration. He found leukocytes near the scars of healed ulcers as late as five months after the use of radiation.

Phagocytes laden with cell debris are common in many reactions, and Fromme found marked hemosiderosis in the intestines of irradiated guinea pigs.

(*d*) Lymph follicles: There is early and marked alteration in the lymph follicles (Engelstad, 1935; Dawson, 1925). Most workers have noted this, describing varying degrees of cellular destruction which lead to complete disappearance of the follicles. The formation of new follicles after such destruction is rapid. Most observers consider (Tsuzuki) lymphocytes the most sensitive elements in the intestine. Warren and Whipple (1922 a), however, found evidence of only moderate injury to follicles in intestines showing marked epithelial damage.

(*e*) Muscle: An alteration of special interest is a rather characteristic vacuolation of muscle fibers.[18] This is recognizable in the gross as fine translucent striations (Warren and Friedman). Nuclear vacuolation and irregularity are also present (Podesta; Regaud and co-workers). Hyaline changes in the contractile material of the fibers, with edema and

14. Regaud and others. Lazarus-Barlow. Warren and Friedman.

15. Delbet and others. Regaud and others. Ghilarducci. Tsukamoto. Brocq and Oury.

16. Martin and Rogers (1923). Terplan. Schürch and Uehlinger. Warren and Friedman.

17. Kolodny. Lawrence and Tennant. Lazarus-Barlow. Dawson (1927).

18. Martin and Rogers (1923). Delbet and others. Wolfer. Lazarus-Barlow.

gelatinous changes of the intermuscular stroma, were described by Lazarus-Barlow. Atrophy of muscle fibers occurs with higher doses of radiation (Warren and Whipple, 1923 g).

Although Engelstad (1935) and Schürch and Uehlinger attributed the changes in the muscle to the local ulcerating process, the changes have been seen repeatedly beneath an intact mucosa. It is obvious, however, that necrosis and inflammatory reaction in the muscle accompany ulceration.

(*f*) Nerve: Degeneration of the nerve cells of the myenteric plexus was described by Colwell and Gladstone, but Engelstad (1935) found no such change in ganglion cells in the stomach.

(*g*) Serosa: Desquamation of the serosa was noted by Lazarus-Barlow. Schürch and Uehlinger, using local implantation of radium, described a specially marked reaction of the subserosal connective tissue, and Warren and Friedman found a similar reaction near gold radon seeds. This response is probably due to the local intensity of the gamma rays. Engelstad's (1935, 1938) failure to find this change may therefore, have been due to the fact that he used roentgen rays.

Mucosa of Stomach and Intestine.—The changes in individual cells and tissues lead to profound alteration and disorganization of the integrated structures of the stomach and intestine. Sometimes, although the continuity of the surface is preserved, a markedly altered mucous membrane results, within which the atypical cell formation is reflected in atypical gland structures. In other cases the destruction of the surface proceeds to ulceration with all the secondary changes, such as infection, that accompany it.

(*a*) Gastric mucosa: Most workers agree that the glandular epithelium is more easily damaged than the surface epithelium.[19] A few observations to the contrary are recorded, however. Lazarus-Barlow found that desquamation of the investing epithelium took place with smaller doses than were required for desquamation in the glands. Brocq and Oury, working with guinea pigs, described disseminated necrosis in both surface and deep layers while the middle zone was spared. Other workers found that damage of the mucosa ranged from destruction of a superficial zone to involvement of the entire thickness (Dawson; Snell and Bollmann).

Although all cell types show damage, some types are more prone to destruction than others. The chief cells show degenerative changes with a dose as small as 500 r (Engelstad, 1938). Many workers, who agree that the chief cells are the most susceptible, observed that in some experiments these were almost wiped out while other cell types,

19. Werner. Brocq and Oury. Delbet and others. Regaud and others. Schürch and Uehlinger.

such as the parietal cells, were preserved. Dawson stated that the gland neck cells and the mucous cells were more easily damaged than the chief cells and that the parietal cells were the most resistant. Delbet, Herrenschmidt and Mocquot found the mucous cells to be the most resistant.

Regaud, Nogier and Lacassagne found that the pyloric glands were relatively unaltered in dogs despite changes in the fundus. Brocq and Oury concluded that dogs showed more marked changes in the pylorus, and guinea pigs, in the fundus. Their protocols seem to show, however, that definite changes took place in both regions. Engelstad (1935) also described damage to pyloric gland cells.

The mucosa may present a strikingly bizarre appearance as a result of various combinations of atrophy, proliferation, metaplasia and fibrosis.

Atrophy of glands, with reduction of the thickness of the mucosal surface, is common. As the chief cells especially are damaged, the glands may be lined by parietal cells.[20] Snell and Bollmann found marked atrophy in the gland necks. The destruction of glands sometimes leaves only scattered remnants of cell nests, composed of atypical cells and showing active mitosis (Delbet and co-workers; Tsukamoto). At times narrow glands lined by flat cells give the mucosa a peculiarly distorted, elongated appearance (Warren and Friedman).

Not uncommonly the glands become irregularly shaped, with wide lumens approaching cystic proportions, lined with atypical cells and sometimes filled with desquamated cells, leukocytes or debris.[21] Papillary structures were seen by ten Doornkaat Koolman in such cystic glands.

Another feature of such a mucosa is the presence of considerable fibrosis, with an increased amount of connective tissue separating the glands.[22] This occasionally reaches marked proportions, sometimes with the participation of inflammatory cell infiltration, and has been described as an "interglandular cirrhosis."

Atypical proliferating epithelial cells are common near ulcers, and because they appear to participate in the healing process they have been considered as reparative and regenerative forms.[23] Engelstad (1935, 1938) found that they were a prominent feature in some of the animals studied during the later healing stages, about two to five months after exposure to radiation. Numerous glands and surface structures

20. Delbet and others. Szego and Rother. Brocq and Oury. Dawson (1925).

21. Delbet and others. Regaud and others. Szego and Rother. Brocq and Oury. Engelstad (1938).

22. Regaud and others. Brocq and Oury. Engelstad (1938). Snell and Bollmann. Szego and Rother.

23. Delbet and others. Aue and Ceculin. Wolfer.

composed of mucous cell types are described, some reaching markedly hyperplastic papillary proportions.

(*b*) Intestinal mucosa: The greater vulnerability of the epithelium of the intestinal crypts in comparison with the surface or investing epithelial layer has attracted considerable attention.[24] The earliest detectable histologic evidence of cell degeneration appears in the glands and crypts almost immediately after these have been irradiated. With desquamation of the surface epithelium, the villi sometimes persist as naked stalks. When they are wiped out (Regaud and co-workers; Ivy and co-workers, 1924), the crypt cells, although exhibiting alterations, may be preserved (Ivy and co-workers, 1924; Dawson, 1927).

The persisting glands may show cystic dilatation[25] resembling that seen in the stomach, frequently with atypical cell formation. Atrophy and fibrosis of the mucosa are also seen (Regaud and co-workers).

Regenerative and reparative attempts are similar to those described in the stomach. Lawrence and Tennant found marked destruction of the mucosa in the first few days after it had been irradiated with neutrons, but described recovery and regeneration taking place as early as five days.

(*c*) Gastric ulcers: Desquamation and erosion of damaged mucosa are observed frequently and lead to frank ulceration. Mucosal degenerative alterations are commonly most striking in the region of ulceration. Discrete ulcers have been produced in rabbits, dogs and guinea pigs by either radium or roentgen rays,[26] most often in the pylorus and along the lesser curvature. They may be limited to the mucosa, although many penetrate the muscular coats. Perforation occurred in approximately one third of the ulcers in some series,[27] and hemorrhages were not uncommon. Ulcers occurred as early as one to two weeks after exposure to radiation, but most of them were found after about one or two months (Delbet and co-workers; Engelstad, 1938). Zones of necrosis, inflammation and fibrosis were present in the ulcer base.

Scarring and healing were found by Engelstad (1938) to begin about the second or third month after radiation treatment. He stated that the high incidence of perforation before this interval of time had elapsed might be due to the inhibition of connective tissue proliferation until this period. Wolfer found that cicatrization took place from two hundred

24. Regaud and others. Hall and Whipple. Warren and Whipple (1922 b). Tsuzuki. Terplan. Lawrence and Tennant. Schürch and Uehlinger. Podesta. Warren and Friedman.

25. Ivy and others (1924). Dawson (1927). Schürch and Uehlinger. Warren and Friedman.

26. Delbet and others. Ghilarducci. Wolfer. Aue and Ceculin. Schürch and Uehlinger. Engelstad (1928). Hayashi and Maruyama.

27. Aue and Ceculin. Schürch and Uehlinger. Engelstad (1938).

to six hundred days after exposure to radiation. He compared the lesions with "peptic ulcers" and thought they showed less scarring than the latter. Aue and Ceculin described actual stenosis and the formation of the hourglass type of stomach. Tsukamoto and Szego and Rother also found considerable growth of connective tissue in ulcerated mucosae. In contrast to these observations, Schürch and Uehlinger failed to find much evidence of scarring in ulcers produced by radium.

The presence and importance of vascular changes in relation to these ulcers have been given much attention. The general conclusion appears to be that although some lesions in vessels have been found, the ulceration cannot be attributed to anatomic interference with the blood supply. Schürch and Uehlinger, Ghilarducci, and Engelstad (1938) found occasional vascular lesions. They described endophlebitis, endarterial thickening and even thrombosis, but they all commented on the scarcity of such lesions in the ulcers. Wolfer, however, although he mentioned the scarcity of blood vessels in the fibrous ulcer base, believed that there was more in the way of vascular change than is seen in peptic ulcers. He described endarteritis and hyaline change in vessel walls. Aue and Ceculin thought that the vascular sclerosis they found was quite comparable to that seen in peptic ulcers.

Engelstad (1938) injected the blood vessels of the ulcerated stomachs of 5 animals in his series. In 1 animal a roughly wedged-shaped area failed to fill with the injection material. He explained this infarct-like area on the basis of edema, since it could not be correlated with demonstrable vascular lesions.

Hayashi and Maruyama attributed the gastric ulcers produced in guinea pigs to ischemia resulting from marked contractions of the stomach musculature. They expressed the belief that the excessively strong contractions were due to a state of hypersensitivity.

(*d*) Intestinal ulcers: So-called late or delayed reactions, especially chronic ulcers and cicatrizing lesions, have been produced in the intestine by many workers. They are not as common as those seen in the stomach, possibly since doses of sufficient magnitude administered over the intestine are more often fatal than similar ulcer-producing doses over the stomach.

Perforating ulcers are found as early as nine days after the tissues have been irradiated, but usually discrete local lesions do not appear until one to four months.[28] They occur in all portions of both the small and the large intestine and range in extent from small lesions extending over only a portion of the bowel circumference to annular lesions. The ulcerative process may extend along the intestine for some distance. "Chronic perforation" with granulation tissue reaction in the surround-

28. Regaud and others. Martin and Rogers (1923). Ivy and others (1924). Podesta. Schürch and Uehlinger. Lazarus-Barlow. Warren and Friedman.

ing structures leads in some cases to complete destruction of the original bowel wall and the formation of a sinus tract conveying the fecal stream (Warren and Friedman).

In many of the instances of ulceration which have been recorded, sufficient scarring took place to cause stenosis of the lumen of the intestine. Martin and Rogers (1923, 1924) also described marked shortening of the involved loops. On the other hand, several workers have commented on the relatively poor granulation tissue response and the lack of healing and scarring in these ulcers (Schürch and Uehlinger). Inflammatory cell reactions have been varied and the vascular lesions relatively insignificant.

Functional Effects

Gastric Secretion.—Most of the experimental studies on the influence of radiation on gastric function have dealt with the effect on secretion (Desjardins; Palmer and Templeton). They were made on both gastric fistulas and Pavlov pouches in dogs, and the doses used were of the same order of magnitude as those required to produce definite histologic alterations. The major effect consisted in a depression of gastric acidity.

The depression and suppression of the secretion of acid is evidenced by lowering of free and total acidity, sometimes with lowering of the volume of secretion or alteration of the fluid to almost pure mucus (Ivy and associates, 1923). Snell and Bollmann found that shortening of the duration of secretory activity was important in diminishing the total volume of secretion. The effect begins as early as a few days after exposure and persists for about two weeks to four months (Ivy and co-workers, 1923; Miescher).

A return to normal concentrations of acid usually takes place, although the volume of secretion in the recovery phase is sometimes reduced. Snell and Bollmann found that after recovery from the effect of radiation, subsequent irradiation of the mucosa was less effective in depressing acidity.

Evidence of so-called stimulation of secretion in the form of an elevation of acid content has been offered. Some of these studies, of which the report of Szego and Rother may be taken as an example, show protocols inconsistent with the conclusions drawn. The considerable variation in values from test to test was over such a wide range that the supposed alterations after exposure to radiation were well within such limits. In other cases, extragastric effects may have confused the picture. In some of Ivy's experiments, for example, in which the whole abdomen was irradiated, intestinal damage was produced (Ivy and associates, 1923). Immediately following the exposure to radiation a transitory rise in acidity preceded the depression. No such effect was

observed following direct irradiation of the stomach alone. Ivy thought that pylorospasm, or increased irritability of the secretory mechanism, possibly related to intestinal damage, might be responsible for the temporary rise.

Case and Boldyreff found that in the "psychic phase" of secretion studied by means of sham feeding, radiation cut down the quantity of secretion without altering its acidity. In the "chemical phase", studied in a gastric pouch, acidity was unchanged, although the quantity was diminished and its appearance delayed. They also believed that intestinal damage might account for this.

However, the results of the most clearcut experiments [29] have been in agreement that irradiation of the stomach results in depression of acidity.

Aue and Ceculin, who used local radium implants, found no effect on secretion and acidity, probably because the radiation was not generalized over the entire stomach. In one of Ivy's dogs normal secretion of acid took place in a portion of the stomach that was not irradiated, while in the irradiated region the acidity of the secretion was depressed (Ivy and associates, 1923). This is fairly conclusive evidence for a local rather than a systemic effect of radiation.

Several studies of the pepsin content of gastric secretion following irradiation of the stomach showed a depression that lagged behind the depression of acid both in its onset and in its recovery,[30] although in one experiment (Case and Boldyreff) an increase in pepsin was reported.

It is extremely interesting that, although anatomic studies show that the major damage after treatment by radiation takes place in the chief cells, physiologic studies indicate that the secretion of acid, formed by parietal cells, is the more strikingly altered. Dawson (1925) found normal-appearing parietal cells despite marked depression of acid.

A few other observations of the effect of radiation on the gastric secretion have been made. Ivy and co-workers (1923) found that the chloride concentration was unchanged. No definite conclusions regarding rennet can be drawn from a few scattered measurements.

Intestinal Absorption.—The absorptive behavior of the intestine following irradiation of the abdominal region shows pronounced alterations. Buchwald found that the amounts of dextrose, fructose and mannose absorbed are cut approximately in half, although the ratios of absorption of these sugars are unchanged.

Impaired and altered fat absorption has also been described. Mottram, Cramer and Drew found alterations in the appearance of the intracellular fat droplets in the intestinal epithelium. With higher doses,

29. Portis and Ahrens. Miescher. Ivy and others (1923). Snell and Bollmann.

30. Portis and Ahrens. Miescher. Ivy and others (1923).

absorption and transfer of fat stopped completely. They noted some resemblances between these phenomena in irradiated animals and those in animals inadequately supplied with vitamins. Martin and Rogers (1923, 1924) gave dogs a meal high in fat and found no fat in the lymphatics of bowel loops one week after irradiation of the abdomen, although control loops showed considerable fat. This effect followed a dose of radiation smaller than that required to cause desquamation and ulceration. Dodds and Webster found an increase in fecal fat, especially in the neutral fat fraction, in human beings after irradiation of the abdomen.

Resistance to Bacterial Invasion.—The effect of irradiation of the intestine on the permeability, or more properly the penetrability, of the mucosa to infecting organisms has been an interesting problem to many investigators. Many workers found bacterial masses clumped against the damaged epithelial surfaces, and some found bacteria in deeper tissues, such as the submucous lymphatics. Organisms presumably of intestinal origin, such as Bacillus coli, have been recovered from blood cultures after irradiation of the intestine.[31]

Warren and Whipple (1923 d) found that the heart blood of heavily irradiated animals was sterile except during the terminal agonal state, although organisms were also found in the mesenteric nodes and other viscera. Chrom found general invasion by organisms after exposure of the entire body to radiation and extension only to local structures, such as the mesenteric nodes, after local exposure. He found that shielding of the liver and the spleen from radiation prevented the general invasion. Lawrence and Tennant found organisms in the blood of animals surviving small doses of radiation. They failed to find organisms in the blood of animals dying after doses that were rapidly fatal, although there was an agonal invasion of the blood stream by bacteria in this group.

These data cannot be clearly interpreted because effects on the body as a whole as well as on the intestine are involved. Systemic resistance was found to be lowered by the radiation in some of these experiments (Chrom). The protocols of other experiments present similar evidence to this effect (Mottram and Kingsbury).

Perhaps the most unequivocal experiments are those of Bonanno. He found precipitins to human serum in the blood of animals after oral administration of serum followed by exposure to radiation. He was able to elicit anaphylactic reactions as well. Agglutinins against typhoid bacilli, and increased resistance to infection by this organism, were produced with a similar technic.

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31. Keller. Warren and Whipple (1923 d). Mottram and Kingsbury. Chrom. Lawrence and Tennant.

It may be of further interest to point out that Mottram described the presence of protozoa (Trichomonas) within the intestinal epithelial cells of irradiated animals. Warren and Friedman found exacerbation of coccidiosis in rabbits after irradiation of the abdomen with doses of 4,000 r. Honess recently suggested the use of roentgen rays in small doses (450 r) as a therapeutic agent in coccidiosis.

Miscellaneous Effects.—Increase in the tone and in the peristaltic activity of the gastrointestinal tract has been reported after irradiation both of intact animals and of isolated loops.[32] The cause and the significance of the increased autolysis of the mucosa after exposure to radiation (Warren and Whipple, 1922 c) have not been determined.

THE STOMACH AND THE INTESTINE: CLINICAL STUDIES

The Intestine.—Ulceration of the intestine is the fundamental process in radiation enteritis. It may be irregular and even generalized, but more often it is focal and discrete. Progressively destructive lesions lead to perforation and the formation of fistulas, and productive cicatrizing lesions lead to stenosis and obstruction. A sufficiently widespread process may terminate fatally before it has progressed far.

The "delayed" reactions are seldom pure radiation lesions, trauma and infection playing roles in their evolution and progression. Most of the lesions can be considered only in the category of chronic ulcers, since few studies of the finer cytologic and mucous membrane effects, such as have been seen in animals, are available.

The great majority of the intestinal radiation reactions seen in human beings occurred in women after therapeutic irradiation of the generative organs. However, perfectly comparable lesions are seen after irradiation of any part of the abdomen and may follow the use of any form of radiation. With modern methods, technical errors of improper filtration, overdosage, displacement of applicators and the like are rarely responsible for their occurrence. However, the methods of administration of radiation are of the utmost importance. Increased fractionation of the total dose may cut down the incidence of intestinal damage.[33]

Immediate Reactions.—(*a*) Simple enteritis: Diarrhea and cramps are common following irradiation of the abdomen and pelvis, and examination of the rectal mucosa may show hyperemia, edema and exudation of fibrin, with a granular surface that bleeds readily ("granular proctitis") (Todd). This mild reaction rarely persists

32. Swann. Toyoma. Andersen and Kohlmann.

33. Anselmino and Oehlke. Corscaden and others. Standardization Committee of the American Radium Society.

beyond two to three weeks after exposure [34] and occurs in 20 to 30 per cent of persons receiving radiation to the pelvis.[35]

Some observers believe that the patients with the more severe and more prolonged early reactions, particularly those accompanied by bleeding and lasting more than two to three weeks after exposure, are likely to have delayed reactions (Collins and Jones). Stark (1924 b) stated that no "late" reaction takes place in the absence of an "early" reaction. Wille, on the other hand, felt that the development of late complications could not be predicted.

(*b*) Generalized ulcerative enteritis: Multiple ulcerations of the bowel are not uncommon in radiation reactions. However, they may be so diffusely spread throughout the intestine in the immediate post-radiation period that they present a clinical picture of severe bloody diarrhea and marked constitutional symptoms. This form of immediate reaction may be fatal, but fortunately is rare.

In a case reported by Cathie the patient died seventeen days after radiation treatment, and other workers have reported instances of death occurring in three to six weeks.[36] In the early days of radiation therapy such deaths were sometimes attributed to technical errors, with extreme overdosage. However, in some of the cases cited the patients were treated with only moderate doses.

So striking were some of these reactions that Seitz and Wintz suggested that 2 of their cases, and 1 of Franz and Orth were instances of coincidental dysentery.

Delayed Reactions.—Delayed reactions, so-called late or chronic lesions, are far more frequent and have been reported by the score.[37] As has been stated before, they are essentially ulcerative processes and the sequelae of ulceration. The clinical symptoms usually appear some weeks or months after exposure, usually between six and twelve months after exposure, although they may occur years later (Todd; Buie and Malmgren).

The frequency of such lesions varies in different series. The incidence of ulceration, stenosis and fistula formation has become less

34. Newell and Crossen. Bailey. Bensaude and others.

35. Corscaden and others. Rahausen and others. Ward. Anselmino and Oehlke.

36. Todd. Ball. Warren and Whipple (1923 h). Schmidt. Buhtz.

37. Krecke. Kroemer. von Franque. Eckelt. Heck. Kolde. Béclere. Benthin. Haendly. Fischer. Mathias. Fried. Mühlmann and Meyer. Schwarz. Holfelder. Stark (1924). Sanders. Glaser. Heidler. Jayle. Jeanneney and Wangermez. Siegmund. Rullé. Schilling-Siengalewicz. Buie and Malmgren. Flaskamp. Bensaude, Oury and Godard. Emery. Kleine. Malmgren. Jones (1935). Newell and Crossen. Pemberton (1935). Bowing and Fricke. Brust. Bacon. Hinz. Brunschwig and Fox. L. K. Ferguson. Lovelady. Buhtz. White (1940).

with improvement in therapeutic technics over the years. Even with modern methods, however, the figures given range from about 1 to 5 per cent.[38] Higher percentages are recorded when less severe reactions are included.

The distribution of the lesions in the various portions of the bowel is weighted because of the preponderance, in almost all the series, of patients receiving radiation to the pelvis. Most of the lesions are found in the rectum and sigmoid although other portions of the large intestine, such as the cecum, are frequently involved. Lesions of the small intestine make up a small proportion of the total.[39] Here, the ileum is most frequently involved.

(*a*) Ulcerating and necrotizing type: Ulcers may develop at any point in the large or the small bowel, but the most common sites are the rectum and the sigmoid. The ulcers may be single or multiple, localized to one wall of the intestine or annular. A clearly demarcated ulcer is sometimes very sharp-bordered, with a pseudomembranous necrotic base, and is surrounded by telangiectatic vessels and inflamed mucosa. It may be superficial or it may penetrate the muscularis and perforate. Superficial ulcers heal with sometimes only mucosal atrophy as a residuum. The healing of deeper ulcers, however, is generally associated with cicatrization and the development of strictures.

Perforation into the free peritoneal cavity is not uncommon. Some reports describe the development of fistulas into neighboring organs and even to the exterior, through the skin.[40] Fistulas may be merely tiny communicating tracts, or they may be extensive, forming huge cloacae.

Necrosis is occasionally so widespread that segmental gangrene of a portion of the intestine, rather than a localized ulcer, results. This type of reaction is seen not infrequently in the small intestine and may cause obstruction. The intestine may be rigid and pipelike, resembling the bowel in terminal ileitis, with induration and thickening frequently involving the mesentery (Cabot case). There is often discoloration of the involved tissue with a characteristic pale appearance of the serosal surface (White, 1940). A greenish tinge, even in fresh surgical material, is common in necrotic or hyalinized tissue (Warren and Friedman).

(*b*) Stenosing and cicatrizing type: Lesions which cause partial or complete obstruction, although they are somewhat less common than the ulcerative type of reaction, include almost all the other types of radiation effect. The dividing line between the two types of lesion is

38. Buie and Malmgren. Todd. Ward. Findley. Rahausen and others. Corscaden and others. Collins and Jones.

39. Jones (1935). Pemberton (1932). Haendly (1921).

40. Wetzel. Warnekros. von Franque.

more on clinical than on pathologic grounds since so many lesions present ulcerative and cicatricial processes at one and the same time. Functional stenosis may be present in a segment the lesion of which is essentially ulcerative and necrotizing, such as a gangrenous loop.

Many strictures are the end result of the scarring and contraction which follow mucosal damage and ulceration. There is, however, one type of stenosis which develops despite the absence of preceding mucosal damage or ulceration. This type occurs in the rectum and has been described as an "extrinsic" lesion (Todd), periproctitis, perirectal stenosis (Berard and Creyssel) and pelvic cellular sclerosis (Rahausen and associates). It is due to contraction of the extrarectal and perirectal tissues, with stricture of the rectum resulting. The uterosacral ligaments, the parametrial tissues and the rectovaginal septum (Mugniery) may be involved, and the lesion may be felt as a crescentic constricting band impinging on the anterior wall of the rectum. On the other hand, involvement of all the pelvic connective tissues simulates a "frozen pelvis."

(*c*) Miscellaneous special types: There is a higher incidence of damage to the intestine after irradiation of the cervix following supracervical hysterectomy than after irradiation of the intact uterus (Ward). The formation of enterovaginal fistulas (Counseller and Foor; Phaneuf), as well as other types of reaction, (White, 1940), is reported. The presence of adhesions binding a loop of bowel to the cervix receiving radiation has been regarded as explaining the increased vulnerability of the intestine. This concept has been extended by those writers who attribute all types of intestinal lesions to the preexistence of adhesion fixing a loop of bowel in the pelvis. This is an extremely important point and will be discussed later.

Radiation lesions develop readily in tumor-infiltrated tissues.[41] This is especially true with regard to fistulas, particularly those of the rectovaginal type. It has been shown (Smith, 1938; Strachan) that fistula formation may occur more often in far advanced cancer which has remained untreated than in irradiated cancer.

Several workers have gone so far as to state that most radiation lesions in the intestine occur as a consequence of damage to tissue infiltrated by tumor. That radiation lesions arise in such tissue is undeniably true, but certainly not the great majority of radiation lesions.

Among other curiosities of radiation effect, the case of Salvesen and Kobro should be cited. They reported an instance of symptomatic sprue in a woman in whom two strictures of the small intestine were found. She had received radiation therapy eighteen years before.

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41. Bensaude and others. Bailey. Hinz. David. Ward. Buie and Malmgren. Elliott and Jenkinson.

Hamperl reported an instance of pseudomembranous colitis following oral ingestion of a preparation of thorium X. A direct effect of the thorium on the mucosa cannot be denied, but since marked leukopenia developed the enteritis may have been due in part to this. Hemorrhages in the large intestine as well as in other organs after the administration of thorium preparations have been reported by Gudzent and others.

Histologic Observations.—Since the essential lesion is ulcerative, even when a cicatrizing process predominates, secondary inflammatory processes almost invariably confuse the picture. Necrosis and inflammatory cell infiltrations of every description are common. However, certain histologic pictures, though not specific to the extent of being pathognomonic, are specific enough to be highly suggestive of radiation effect.

(*a*) Mucosa: The variety of lesions seen in animal experiments has not been described in human beings. Erosion and ulceration with destruction of the surface epithelial layer are common. Atrophy of the mucosa was described by Fischer and by Case and Warthin, and the latter authors noted "mucoid degeneration" in the surviving epithelium. Sanders found a single-layered regenerating epithelium, and epithelial cell proliferations resembling those seen in animals have been observed (Warren and Friedman).

(*b*) Connective tissue: The typical changes in connective tissue have been described by many workers. They are important in the histologic diagnosis of radiation reaction.

The swelling, or hyaline change, of the collagen with a resulting afibrillar, poorly cellular matrix is highly characteristic (Fischer). This so-called fibrosis takes place in the mucosa, submucosa, muscularis and serosa and in the walls of blood vessels. It is accompanied frequently by bizarre, giant fibroblasts.[42] The subserosal connective tissue frequently shows this type of alteration. The changes in the connective tissue may be presented in ulcerated bowel as well as beneath a preserved mucosa. Occasionally they have been confined to swollen, clublike villi (Warren and Friedman). Many writers have noted that in man, as well as in the experimental animal, there is a lack of organization and active granulation, and that proportionately little true fibrosis takes place.

(*c*) Vessels: The alterations in blood vessels and lymphatics have attracted considerable attention. Hemorrhages, hyperemia and thrombosis are common, as well as the more specific telangiectasia, but structural changes in the vessel wall have received the greatest emphasis.

Intimal and medial thickening and perivascular adventitial fibrosis with hyaline and degenerative changes in the connective tissue have all

42. Fischer. Sanders. Cabot case 25252.

been described.[43] Case and Warthin described endothelial degeneration. Changes in veins and venules, more marked than in arteries and arterioles, were described by Fischer and Buhtz. Although Sanders and Fischer found that the elastica participated in this endophlebitic process, Buhtz disagreed with this observation.

Cathie found undamaged blood vessels in cases of immediate reaction despite ulceration, and Pemberton failed to see obliterative endarteritis. Even Fischer described the presence of many normal as well as altered blood vessels, while Buhtz, Sanders, and Mühlmann and Meyer found changes in vessels in the intact, nonulcerated portions of the mucosa. Todd, on the other hand, expressed the belief that the endarteritis observed was responsible for the development of rectal radiation lesions. The "extrinsic" lesion in particular was described as an "infarction" of the pelvic cellular tissues under the influence of radiation.

However, the human material in general is similar to the experimental animal tissues in its failure to provide definite evidence that the ulcerations are due primarily to obliteration of the blood vessels.

(*d*) Muscle: The muscle coat presents some interesting lesions, apart from the extension of an ulcer and the accompanying processes of necrosis, inflammation and scarring. Atrophy of fibers (Case and Warthin) may be quite irregular, and in some material (Warren and Friedman) together with fibrosis a striking picture of "myofibrosis" was seen. Vacuolation and nuclear destruction were found by Case and Warthin.

The hypertrophy described by some workers (Buhtz; L. K. Ferguson), rather than being a direct radiation effect, may have been due to obstruction.

(*e*) Other tissues: Atrophy of lymphoid tissue has been commonly described, and ganglion cell degeneration was observed by some workers. Marked serosal reactions, especially of the type of the connective tissue changes described, with associated telangiectasia are common. The inflammatory reactions of perforation and peritonitis are self evident.

The Stomach

Pathologic Changes.—Studies of the changes consequent to irradiation of the human stomach from the point of view of pathology are relatively few. Ulcers and perforations of the stomach following exposure to radiation have been reported. These occurred in some instances in stomachs infiltrated with tumor, where destruction of tumor tissue may have been primarily responsible.[44] In Schönleber's case, reportedly similar, there was peritonitis but no demonstrable perforation.

Case and Warthin studied the stomachs of 3 patients who had received radiation for gastric tumors. They found atrophy of glands,

43. Case and Warthin. Pemberton (1932). Buhtz. Heck. Sanders.

44. Wetzel. Schmitt. Elliott and Jenkinson.

mucoid and vacuolar degeneration of cells, and necrosis with ulceration in portions of the stomach not involved by tumor.

Humphreys studied the irradiated stomachs of 2 patients in the Palmer and Templeton series. One stomach at the completion of a course of 3,200 r showed cellular degeneration with nuclear pyknosis and vacuolation and giant syncytial forms. The other, six months after a course of 2,900 r, showed fibrosis with glandular atrophy and numerous abnormal glands of the mucous type. Many of the alterations were most marked at the level of the gland necks, and the chief cells showed marked changes despite the presence of normal parietal cells.

Recently Warren and Friedman studied the changes in a stomach removed at operation one week after treatment with 5,400 r. It showed an ulcer, probably related to the regression of a neoplastic process. There were numerous glands of the mucous cell type in regenerating mucosa at the edge of the ulcer. Cell destruction was most marked at the level of the gland necks. Cystic and elongated, distorted, atypical glands were numerous. The most unexpected and striking change, however, was seen in the intact mucosa at some distance from the ulcer. There was an extreme degree of vacuolation of the parietal cells while the chief cells appeared to be entirely normal.

Functional Changes.—No attempt will be made to do more than mention briefly the host of observations on the effect of radiation on the functioning of the human stomach (Desjardins; Palmer and Templeton). Most of the studies have dealt with alterations in gastric acidity in normal persons and in patients with hyperacidity or peptic ulcers. Radiation has also been employed therapeutically for spastic conditions of the stomach, for hypoacidity and in attempts to alleviate polycythemia (Andersen; Stenstrom and co-workers).

There is little doubt that irradiation of the stomach with doses within the ordinary therapeutic range results in a fairly prolonged, if transient, depression of acidity lasting some months. Both free and total acid have been depressed, and achlorhydria has been produced. There have been scattered reports of "stimulation," similar to those mentioned in the experimental section, but the same criticisms and doubts expressed there are valid here. The reality of the depression and suppression, however, seems to be beyond doubt.

As in the experimental work, pepsin has been depressed as well as acid, but this effect has been less marked than the effect on acidity.

GENERAL COMMENT ON THE GASTROINTESTINAL OBSERVATIONS

Epithelial Regeneration.—The varied histologic appearance of the regenerative phenomena which are seen in mucosae damaged by radiation are, as Warren and Whipple have said, a delight to the pathologist. The

"mucous change," the giant and syncytial forms and the bizarre atypical epithelial cells have been discussed in some detail. It should be emphasized that these changes are not specific radiation effects. They are seen in miscellaneous disease processes of the stomach and intestine and also as a result of experimental procedures other than irradiation of tissues.

The presence of a more marked effect of radiation in the cells of the intestinal crypts and in the cells of the necks of the glands of the stomach is probably due to the fact that in these regions proliferation of cells takes place.

Increased numbers of mucous cells have been observed [45] in the gastric glands near regions of mechanical injury and in chronic inflammatory processes as well as after exposure to radiation. The "pseudomucinous" change may be due to differences in the staining properties of various types of mucus-producing cells (Bensley; Maximow and Bloom). Dawson (1925) also found two types of parietal cells in the stomach after injury by radiation. The inclusions in intestinal crypt cells described by Dustin and by the Smallwoods were present after suppression of mitosis by either chemical agents or radiation.

The "rejuvenation" cycle which Popoff described involves transformations between parietal cells, mucous cells and argentaffin cells in the absence of mitotic activity and may explain these miscellaneous observations.

Cystic glands are seen in the stomach in a variety of conditions (Popoff, 1941). A resemblance between similar cystic glands observed in the intestine after radiation injury and those described in the intestines of patients with avitaminosis (Denton; Herzenberg) has been pointed out by Martin and Moursund. The formation of syncytia in the intestinal epithelium is described in normal development in some species (Smallwood and Smallwood) and is seen in regenerating intestinal epithelium after various types of injury (Patzelt).

All these observations indicate that most of the histologic changes described as effects of radiation represent nonspecific damage and nonspecific attempts at repair.

Genesis of Radiation Lesions.—The primary effect of radiation on the gastrointestinal mucosa is probably direct. Various theories of indirect action [46] are rarely given credence in current literature. The destructive and degenerative changes in cells that have been described are typical of the effects of radiation on cells in general.

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45. Harvey. A. E. Ferguson. Bensley. Plenk. Popoff (1941).

46. Krause and Ziegler. Keller. Hall and Whipple. Denis and others. Hayashi and Maruyama.

However, secondary factors play a part almost from the beginning, particularly in progressive ulceration. Engelstad (1935, 1938) stressed their role in the development of experimental radiation ulcers of the stomach. He expressed the belief that ulcers develop under the influence of other factors, chiefly mechanical trauma, in mucosa damaged by radiation. The relative infrequency of ulceration in irradiated Pavlov pouches supports this contention. Secondary infection probably plays an even more important part in the intestine than in the stomach. The resistance of the intestine of the fetus as compared with that of the pregnant mother rat should be mentioned here (Colwell and Gladstone, 1937). Although it has been considered that infection predisposes the tissues to damage by radiation, it is more important as a secondary factor.

It has already been pointed out that radiation ulcers are not caused by obliteration of vessels. However, damage to vasculoconnective tissue may influence the course of the lesion. Many workers have commented on the poor healing of radiation lesions. Damaged stroma may be inadequate to support epithelial repair (Ivy and associates, 1924; Wolbach).

The sharp demarcation of the zone of injury has been noticed by several workers. The borders of the lesions were considered by some to be direct projections of the edges of the beam of radiation (Warren and Whipple, 1923 f). This may be true for the initial injury, but it certainly cannot hold for the delayed lesion.

Engelstad (1935, 1938) found that most of the gastric ulcers resulting from irradiation of rabbits were approximately 1 cm. in diameter. Yet he had used a portal that was considerably larger. Obviously the ulcer developed in only a portion of the exposed and damaged mucosa.

Warren and Whipple (1923 g) stated:

> One is impressed by the patchy injury of the intestinal tract noted in dogs dying from a minimal lethal dose of radiation. Larger doses give a much more uniform distribution of the intestinal injury. We may choose to explain this on the basis of intestinal contents, or the position of various intestinal coils in relation to the cone of radiation, or on the score of intestinal activity, etc., but the fact remains that there is no convincing explanation for this observation at present.

The situation may be clarified by realizing that the spatial distribution of the radiation in the tissues need not determine the exact extent of the final lesion. The longer the interval of time after radiation injury at which the lesion is studied the less strict will such a correlation be. Yet the literature abounds with "explanations" of the topography of radiation lesions based on the assumption that the outline of the lesion must represent the outline of the field of radiation.

Another important point involves the relation between the intensity distribution of the dose of radiation and the final lesion. It has been assumed, for example, that an ulcer always develops in that portion of the bowel that has received the highest dose. This is not necessarily so. A delayed lesion may develop in any region that has received more than a "threshold" dose, and a lesion may fail to develop in a portion of the intestine that has received a much higher dose. The location of the final lesion does not necessarily indicate the point of maximal exposure in the intestine any more than it does in the skin. The ulcers developing in radiation burns of the skin may develop at any point in the damaged region and not necessarily throughout the whole irradiated field or at the region of maximal dosage.

In many cases, it is true, a lesion does develop in the portion of intestine that received the highest dose. The anterior rectal wall, which is so close to the cervix, is an especially favorable site for the development of an ulcer. Such an ulcer may develop because of the close proximity of the rectum to locally implanted radium, or because cross fire roentgen ray therapy may focus several beams on the region of the cervix and "catch" the rectum. Other loops of intestine may become dangerously exposed if they are held fixed by adhesions and in close proximity to the source of radiation or to the lesion at which roentgen ray beams are directed.

The importance of adhesions is not denied but it is important to recognize that immobilization of an intestinal loop is not an absolute prerequisite for the development of a lesion. Radiation damage in "free" loops is not infrequently seen.[47] It has been suggested that such loops must have been stationary in the pelvis during the course of radiation therapy. However, they need to have been exposed to only a part of the total dose in order to receive sufficient damage for a lesion to develop. It is not likely that a given loop will lie in exactly the same position day after day during a course of therapy, nor is it necessary to make such an assumption.

There may be several focal lesions in a single stretch of bowel. It has been suggested that there was greater dosage intensity just at the region of each of these lesions. Even though cross fire may have been used, this explanation is not convincing. Furthermore, entirely similar lesions may be produced in experimental animals by the use of a single field of radiation without cross fire.

Experiments have been done (Warren and Friedman) to test the importance of adhesions and immobilization. In rabbits a loop of bowel was fixed to the anterior abdominal wall and the abdominal field radiated.

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47. Corscaden and others. Pemberton (1932). White (1940). Warren and Friedman.

Typical radiation ulcers developed in various portions of the intestinal tract, but the immobilized loop, which had presumably received the highest dose, was not especially prone to the development of ulcers.

Finally, adhesions may develop after an ulcer has progressed to the point of provoking a serosal reaction, and the damaged loop may then become fixed anywhere in the abdomen (Eckelt; White, 1940) or pelvis. This has also been observed in experimental animals (Warren and Friedman).

Diagnosis of Radiation Reaction.—The clinical diagnosis of radiation reaction should be considered in any case in which gastrointestinal symptoms are present following radiation therapy, even after the lapse of some years. The major alternative diagnosis in the majority of instances is that of extension of a cancer. The symptoms may be so alike that radiation lesions of this type in the rectum have been called pseudocancers (Todd; Rahausen and co-workers). Further radiotherapy for this supposed cancer may be disastrous (Lovelady; Malmgren).

The pathologist can be of great help in making the differential diagnosis between radiation reaction and other lesions. However, he should be informed that a radiation reaction is suspected. A pathologic diagnosis of "chronic inflammation" on a biopsy specimen may be correct but is worthless.

The finer cytologic alterations in the epithelial structures are not of great diagnostic value, although they may be confirmatory. The peculiar hyaline change in the connective tissue, which is more easily recognized than described, is of utmost importance. The presence of bizarre, giant fibroblasts supports the diagnosis of radiation reaction. Telangiectasia, thickened blood vessel walls with hyaline change, and bizarre endothelial forms may be helpful. These changes, no one of which is pathognomonic, are in combination strongly suggestive. There are few lesions other than radiation reactions in which the collagen, the fibroblasts and the blood vessels all show the aforementioned changes.

THE ESOPHAGUS

In general, the reaction of the esophagus to radiation is that of any mucous membrane made up of stratified squamous epithelium. Since the response of such a membrane is discussed in detail in chapter XIII, it will not be considered here.

The response of the esophageal musculature and connective tissue is likewise not peculiar to the organ. One point of interest is the possible formation of diverticula (Herzog) following irradiation of tumors in the vicinity of the esophagus. These are traction diverticula, assumably due to the retraction of adhesions resulting from mediastinitis.

THE SALIVARY GLANDS

Observations on the salivary glands are extremely scanty. We have found only one reference, and that on functional activity (Ivy and co-workers) since Desjardin's review.

These glands show less morphologic than physiologic response to radiation, no histologic change appearing even though secretion may be diminished. A common sequel of therapeutic irradiation of the buccal cavity (Case and Boldyreff; Ivy and co-workers) is dryness of the mouth, varying in duration. Swelling of the glands with diminution of secretion has long been noted. When visible damage is done, it is most marked in the acinous cells and small ducts, and the excretory ducts escape even when the dose of radiation has been heavy (von Salis). Degeneration of the epithelium, often with accumulation of mucus, is accompanied by stromal edema (von Salis) and, later, fibrosis (Horowitz) and lymphocytic infiltration (Ivy and co-workers). Regeneration is rapid.

The mucous portions are more sensitive than the serous, and as degeneration advances the serous acini may appear mucous (Lazarus-Barlow). Mucus plugs may occlude the ducts (Lazarus-Barlow). Fatty degeneration may appear in the epithelium of the smaller ducts (Tsuzuki). The blood vessels show hyperemia for a few days (Tsuzuki).

THE PANCREAS

The pancreas is resistant to radiation injury. Almost no reports of damage to it in the course of therapy have been given. In 1909 Ménétrier and co-workers lightly irradiated the pancreas of a diabetic patient and found no change at autopsy. Case and Warthin, describing changes in a patient who died ten weeks after heavy doses of roentgen radiation over the epigastrium, noted that the acinous cells were atrophic and that the duct epithelium showed vacuolar degeneration, necrosis and lymphocytic infiltration. In view of extensive infected ulcers of the duodenum and the periductal reaction in the pancreas, it appears that some of the pancreatic changes may have been due to, or intensified by, infection. With very light radiation treatment (⅛ to 2 erythema doses), the regeneration of zymogen granules in the acinous cells has been disturbed (Hirsch). Tsuzuki produced slight degenerative changes by heavy doses of roentgen radiation but obtained none with lighter doses.

Pancreatic necrosis has been reported (Schürch and Uehlinger) following the placing of radium (600 mg. hr.) in the foramen of Winslow of a dog. Pancreatic fibrosis and necrosis in the dog, induced by implanting into the gland gold radon seeds in dosages ranging from 528 to 1,584 millicurie hours, was reported by Leven.

It has been suggested that insular epithelium is more resistant than acinous. A relative resistance of the insular tissue has been noted in the guinea pig (Capocaccia and Vallebona). In Lazarus-Barlow's experiments with radium (on frogs, rats, rabbits and cats) gamma radiation produced but little change. The islands were unaffected, but with the heavier doses the acinous cells showed swelling of their supporting collagen, vesiculation of epithelial nuclei and some desquamation. The latter two changes are probably not significant. Lethal hypoglycemia with normal or slightly hypertrophic islands follows moderate roentgen irradiation of the rabbit pancreas, while the acinous cells show vacuolation, loss of granules and pyknosis.

Mitoses in insular epithelium following heavy irradiation of the pancreas, with enlargement of the islands and some peripheral regeneration of acinous tissue following its degeneration, were reported by Rosenbaum in hens. However, since he also spoke of transition of acinous to insular epithelium, his interpretation is open to question.

Lethal hypoglycemia has been produced in rabbits by heavy doses of radiation which caused pyknosis, degranulation and vacuolation of the acinous cells while the islands remained normal or slightly hypertrophied (Terbruggen and Heinlein).

With heavy doses of roentgen radiation, Cameron and Flecker produced degeneration of beta cells in guinea pigs within one to four days, followed by mitotic activity at eight days and fairly complete recovery at eighteen days.

While Ivy and co-workers noted a fibrotic pancreas one-sixth the normal size (4.4 Gm.) in a dog which had received 1 erythema dose, this was probably not due to radiation injury since 6 other dogs receiving the same dose and 2 dogs receiving 2½ erythema doses showed no change.

Fisher reported marked fibrosis of an isolated dog pancreas following 3 erythema doses and complete disappearance of the isolated portion following 4 to 5 erythema doses.

THE LIVER

Parenchyma.—The effect of radiation on the liver should be of unusual interest inasmuch as there are two highly specialized kinds of epithelium besides an important endothelial system and vascular and fibrous elements to compare. But there are several difficulties in the way of a knowledge of the sensitivity and reaction of the liver to radiation. Perhaps the most important is the regenerative ability of the liver cells. The evaluation of minimal or sublethal change is uncertain in cells having as rapid a rate of recovery as liver cells. Moreover, the changes described are often so nondescript as to be practically meaningless. In

many instances the value of such diagnoses as "mild cloudy swelling," "hydropic change," "fatty degeneration" or "disappearance of glycogen" cannot always be clearly interpreted from their context, as they are often incompatible with the descriptions. Also, one cannot always be sure of what the changes consist which are designated as "early degeneration." One author may consider physiologic the same appearances another described as pathologic.

Without much conclusive evidence, it has been assumed that the liver as an organ is relatively resistant to radiation injury. Yet there are reports of necrosis of the liver in man resulting from doses of radiation which have not caused a skin reaction or any demonstrable effect on the stomach: In 1 case 40 to 50 per cent of an erythema dose was given in 2 doses directed to carcinoma of the stomach, two months and two weeks before death (Wetzel); in another, 2,965 r directed to the upper part of the stomach over a period of a week through a 15 by 15 cm. portal was followed three weeks later by acute abdominal symptoms, which at operation proved to be due to focal necrosis of the liver (Palmer and Templeton).

One of the most interesting reports on radiation change in the liver is that of Case and Warthin in which are given the gross and microscopic changes following heavy doses of radiation in 3 cases of cancer of the stomach. Extensive nonspecific inflammatory reactions, acute and chronic, were present in the stomach and the liver in each case. The authors accept as evidence of radiation effect the unusual proliferation of bile duct epithelium to form syncytial masses and giant cells which they consider different from those seen in other types of inflammatory reaction. The giant cells in their figures 7 and 8 show distinct inclusions. This hypertrophy of epithelium has been seen in other organs and is one of the few quite characteristic radiation changes. But it is questionable whether one may fairly assume from this that *all* the changes in the liver were initiated by radiation. The authors concluded that the liver cells are more resistant to radiation than the biliary epithelium.

In the course of observations on the functional activity of the liver in cases of diabetes following exposure to mild radiation (Holzknecht units), Ménétrier and associates were unable to find any histologic changes in the liver in 1 case in which glycosuria was marked.

Acute hepatitis in man has been ascribed to the use of colloidal thorium dioxide (thorotrast [48]) occurring after ordinary doses (Whitaker and co-workers) and after twice the usual dose, in this instance, in two weeks (Randerath and associates). Rigler reported 3 cases of

48. Thorotrast is a stabilized colloidal solution of thorium dioxide containing approximately 25 per cent of thorium dioxide by volume (report by the Council on Pharmacy and Chemistry [J. A. M. A. **99**:2183, 1932]).

primary carcinoma of the liver among 35 patients who had received colloidal thorium dioxide (thorotrast) for diagnostic purposes and on whom autopsies were later made. The length of time which intervened in these 3 cases is not clear. The possibility of the radioactivity of thorium dioxide being responsible for these pathologic changes is probably slight, since the usual dose of 75 cc. given intravenously in 25 cc. amounts daily contains a quantity of thorium dioxide equivalent in alpha ray activity to 1.5 to 3.0 micrograms and in gamma ray activity to 0.9 to 1.2 micrograms of radium.

Because heavy doses of radiation are not commonly directed to the stomach or liver region for therapeutic purposes, pathologists must rely largely on animal experimentation for information. Some of the experiments on animals were done for the single purpose of studying reactions in the liver, while others related to side issues of more general subjects, as radiation sickness. There is naturally a wide range in the conditions of the experiments and the species used and consequently in the hepatic response. The liver of a mouse receiving radiation over the entire body would not react as a dog's liver exposed to roentgen rays outside the body wall even though the factors of radiation were comparable. These variations in radiation factors, species and intervals of time for study of the liver must be constantly kept in mind in drawing conclusions.

Reports of observations on the liver after its exposure to radiation may be divided into three groups: (*a*) those in which no changes, (*b*) those in which slight changes and (*c*) those in which parenchymal areas of necrosis are stated to have been observed.

In some instances there was no visible hepatic response following moderate or even heavy doses of radiation in the dog (Hall and Whipple; Warren and Whipple), the rabbit (Hudellet; Heineke), the guinea pig (Seldin; Heineke) or the mouse (Heineke), even though in some of these instances the animal died in a few days as a result of its exposure to radiation. In some instances the radiation was given in one or two doses; in others over a period of three (Seldin) to twelve weeks (Hudellet). Contrasting irradiated and shielded portions of the same liver after moderate doses had been given, Seldin found no differences between them five weeks later.

Minimal changes and early degeneration have been described under a great variety of conditions after moderate (Tsuzuki) and heavy doses of radiation (Hall and Whipple; Ludin; Pohle and Bunting) and with radium (Werner) in several species, and in both newborn (Tribondeau and Hudellet) and adult animals. Ludin irradiated the liver region in 10 guinea pigs and 2 rabbits with large amounts of soft radiation. Those receiving unfiltered radiation daily died in five to seven days, and the only demonstrable changes in the livers were hyperemia and cloudy

swelling. There was less definite change in the livers after the use of filtered radiation. In reports on animals dying soon after receiving heavy doses of roentgen rays, the liver has been described as being normal or showing only slight changes (Hall and Whipple). A progression of early changes with a roentgen ray dosage apparently sublethal to liver cells (although this is not entirely clear from the microscopic description) is given by Tsuzuki, who irradiated the backs of rabbits with doses varying from 20 to 150 per cent of an erythema dose at one time. Following 16 per cent of an erythema dose (thirty minutes) there occurred slight hyperemia and increase in the fat content of Kupffer cells, changes which with higher doses became more marked and were accompanied by vacuolar and granular degeneration of liver cells. After 48 per cent of an erythema dose (90 minutes) glycogen was reduced rapidly, and with 64 per cent of an erythema dose (120 minutes) the Kuffer cells were found hypertrophied and actively phagocytic. Acute parenchymatous degeneration was followed by complete recovery in three and four days after irradiation except in animals receiving lethal doses, in which a certain degree of "atrophy" was found post mortem.

The detailed and carefully controlled experiments of Pohle and Bunting are worth noting inasmuch as they support observations of others based on less clearly specified physical factors. Even after intense irradiation of the hepatic area only mild changes occurred, which were quickly repaired. Three series of rats were used; one series was given 2,000 r in one dose, and the animals were killed for microscopic study of the livers on successive days for a month; one series was given doses of 600, 800, 1,000, 1,500, 2,000 and 2,500 r at 100 kilovolts and killed two, seven, eleven and thirty days after exposure; another series was similarly treated at 140 kilovolts. The factors of the radiation treatments were: an aperture over the liver of 2 by 1.3 cm.; for 100 kilovolts, a focal skin distance of 20 cm. and a 2.0 mm. aluminum filter; for 140 kilovolts a focal skin distance of 45 cm. and a 0.25 copper and 1.0 mm. aluminum filter. The animals were killed without anesthesia, and cell counts were made on standard size fields. The irradiated livers were compared with livers from a control series of untreated rats. Necrosis was not seen in any of the livers. Moderate degenerative changes, hydrops and fat deposition occurred in two cycles, maximal changes were noted first on the second day, and similar but more marked changes occurred from the tenth to the thirteenth day. Atrophy of liver cells was described on the third to fifth days and on the twentieth day. The second reaction tended to be more pronounced than the first. Practically the same changes were found with both moderate and high doses, and there was no difference between the effects of the two qualities of radiation. Recovery was complete by the thirtieth day.

Atrophy and vacuolation of the parenchymatous cells of the liver have been described with large doses of colloidal thorium dioxide (thorotrast) given to animals (Hugenin and co-workers; Pohle and Ritchie). "Toxic changes" have been seen as late as a year after injection, although the liver usually returns to normal except for slight atrophy and fibrosis after weeks or months (Pohle and Ritchie).

The significance of rather indeterminate changes seems to be confirmed by those which have been described actually preceding necrosis in the liver cells. Intensive irradiation with roentgen rays and radium (Theis) causes necrosis of the liver in rabbits (Kolodny), mice (Mills), guinea pigs (Theis) and dogs (Hall and Whipple).[49]

The primary changes—hyperemia, petechial hemorrhage, swelling, disappearance of glycogen and granular degeneration—have been described as occurring immediately after exposure to radiation (Theis; Mills). Necrosis is seen from twenty-four to seventy-two hours later (Theis). Initiation of repair is almost coincident with injury, although complete healing may be delayed much longer than after other kinds of injury (Higgins and Rogers). The necrosis is generally confined to the liver cells, but Kolodny described necrosis of the biliary epithelium somewhat later than that of the liver cells. Kupffer cells are made prominent by the phagocytosed hemosiderin and granular debris (Tsuzuki; Kolodny). Repair proceeds along normal lines: proliferation of bile ducts, liver cells and fibroblasts, organization of thrombosed vessels, and so on. Damage may be protracted either by secondary degeneration occurring later (ten days to two weeks after) or by retarded repair (Bolliger and Inglis; Mills).

Bolliger and Inglis focused attention on delayed or chronic effects of radiation on the liver. The exposed livers of dogs were given 1,800 r to 5,250 r unfiltered radiation and the changes followed by frequent biopsy. In the majority of cases 50 to 100 cc. of 10 per cent dextrose was given intravenously after exposure. The most extensive damage was seen in animals that died soonest after the administration of very large doses of radiation. Fibrosis was noted in some dogs as early as six weeks after exposure. The apparent chronicity of the radiation effect is brought out in the protocols of 2 of the dogs. One dog that died sixteen months after irradiaton still had foci of necrosis in the liver as well as portal fibrosis and calcification in the region of the

49. Hall and Whipple gave lethal doses of roentgen rays over the abdomens of several dogs. Although the animals died within three to nine days, the livers of all except 2 were normal: in the liver of one of these cloudy swelling with fatty change was noted, and in the liver of the other, focal necrosis. The authors ascribed this to bacteremia following damage of the intestine.

gallbladder. Biopsies showed cloudy swelling at three months and seven months, superficial fibrosis at three months and periportal fibrosis at seven months, which became more marked at nine and twelve months. Another dog became jaundiced two months after irradiation, at which time blood sugar, urea and cholesterol were normal, but the congo red test indicated that hepatic function was below normal. Biopsy one hundred and nine days after treatment showed cloudy swelling and the liver contracted with portal fibrosis; at two hundred and twenty-two days there were cloudy swelling, proliferation of bile ducts and portal fibrosis, but no necrosis. Two hundred and twenty-six days after exposure to radiation death occurred with deep jaundice, advanced central necrosis, fibrosis, calcification and bone with marrow in the liver and fibrosis of the gallbladder. Another dog had practically no change in the liver four months after exposure, but two months later, had deep jaundice, dense portal fibrosis and extreme central necrosis.

Even when allowance is made for variability of change in different parts of the liver, these sequential biopsies give evidence of the resistance of the liver cells to radiation injury. With fairly heavy doses damage, at first sublethal, later manifests itself in slow death of the cell.

Necrosis of the liver has been described in animals given twelve to twenty times the usual dose of colloidal thorium dioxide (thorotrast) (Kadrnka).

Biliary Tract.—The type of proliferation of biliary epithelium described fairly frequently in connection with radiation change in the liver suggests a repair reaction such as occurs with any type of inflammation, rather than one initiated by injury of ducts per se. Werner found no changes in biliary epithelium of rabbits that had been intensively irradiated, even with necrosis of the liver cells. Pohle and Bunting stated specifically that heavy doses of radiation do not kill biliary epithelium. Case and Warthin observed in human livers necrosis of biliary epithelium and a type of proliferation with hypertrophic cells and giant cell formation which they felt was typical of radiation reaction. This was preceded by vacuolation, alteration of staining reactions and cell atrophy. Kolodny also saw rapidly proliferating epithelial cells with occasional epithelial giant cells in the livers of rabbits in which death occurred within a few days after heavy doses of radiation had been given. Necrosis of biliary epithelium was seen later than necrosis of liver cells.

In the gallbladder, light to moderate doses produce little change, while heavier doses, still within the range used in therapy, cause congestion, edema, hemorrhage and necrosis of epithelium with inflammatory reaction, maximal about the twelfth day. The reaction is confined to the fundus except with the larger doses, which affect the whole bladder. This is probably not evidence of difference in sus-

ceptibility of epithelium in different parts of the bladder, as the author suggested, but rather a result of the difference in the position of these parts in relation to the body surface (Brams and Darnbacher).

Calcification in the "region of the gallbladder" was found in 1 dog sixteen months after heavy doses of roentgen rays had been delivered to the exposed liver and dense fibrosis two hundred and twenty-six days after a similar employment of radiation (Bolliger and Inglis).

Reticuloendothelial Cells.—Tsuzuki described hypertrophy of Kupffer cells in animals dying soon after treatment with from 20 to 150 per cent of an erythema dose. In the majority of reports no mention is made of the Kupffer cells.

There have been several studies on changes in function of the endothelial cells after exposure to radiation, but most of these have had no histologic correlation.

Diminished phagocytic activity (for graphite) and necrosis (simultaneous with necrosis of liver cells) have been observed in a zone of liver adjacent to implanted gold radon seeds (approximately 1.16 millicuries each) from three to thirty-five days after the implantation. The animals were examined at intervals of from six hours to three months by biopsy. The maximal injury to liver cells and Kupffer cells was seen between the fourth and sixth weeks. Although there was some evidence of recovery at this time, normal activity of phagocytes did not occur until the twelfth week, as against complete recovery by the fourth week around blank seeds in the control series (Higgins and Rogers).

Calò described hyperplasia of Kupffer cells in white mice after moderate doses, which he correlated with increased resistance of animals to tumors engrafted after universal application of radiation.

The numerous observations of human and animal livers after injection of colloidal thorium dioxide (thorotrast) and other thorium compounds, which are taken up almost entirely by endothelial cells, especially in the liver and the spleen, give little (Shute and Davis) or no evidence of injury to the cells (Rigler and co-workers; Yater and co-workers). This absence of effect is largely due to the low radioactivity and the fact that the chief action of thorium dioxide on tissues, as ordinarily administered, is that of a foreign body. The pathologic picture has been described as similar to that produced by the intravenous injection of colloidal silica (Hugenin and co-workers).

Stroma.—Changes in blood vessels and stroma, attributable to radiation, do not accompany sublethal parenchymatous damage. Gabriel claimed to have found changes in the vessels of the liver following radiation treatment even though the parenchyma was normal. Case and Warthin described necrosis of blood vessels in part of a reaction in

the liver which was considered undoubted radiation effect. The branches of the hepatic arteries were hyalinized and showed marked endothelial necrosis and vacuolar changes in smooth muscle. Thrombi, some well organized, have been noted in the vessels in necrotic foci in rabbits that died three to nine days after heavy doses of radiation (Kolodny). Case and Warthin described hyaline change in portal fibrous tissue. Although fibroblastic proliferation has been described in many livers showing severe necrosis, provided the animals lived long enough, this is not clearly a radiation effect. The edema, swelling and fibrosis, described with large doses and occasionally with ordinary doses of colloidal thorium dioxide constitute a possible but doubtful exception.[50] That there is a possibility of change due to continued though very low radioactivity of thorium acting over indefinitely long periods may be admitted inasmuch as sarcoma has been produced by subcutaneous injection of colloidal thorium dioxide (Roussy and co-workers; Selbie).

Summary.—From the incomplete evidence at hand one can draw no definite conclusions as to the sensitivity of the human liver to irradiation. The livers of some species are quite resistant. The histologic changes of the liver following irradiation of that organ are difficult to produce as compared with the profound physiologic disturbances.

The rapid regeneration of hepatic cells and delayed effects make the detection of radiation reaction difficult.

While biliary epithelium shows a reaction characteristic of radiation injury, liver cells do not.

Provided the threshold for damaging the liver cells has been reached, it is probable that there is no close correlation between the dose of radiation and the degree of injury to the liver cells.

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(To Be Continued)

Notes and News

Appointments.—John A. Sexton, assistant professor of pathology at Cornell University Medical College, has been appointed to the same position in the school of medicine of Washington University, St. Louis.

Elson B. Helwig and Edward Smith, instructors in pathology in the Washington University School of Medicine have been given leave of absence for service in the United States Army as captain and lieutenant, respectively.

Carl W. Apfelbach, Rush professor of pathology at the University of Illinois College of Medicine, has been appointed medical director of the Presbyterian Hospital, where he has served as pathologist.

In the University of Pennsylvania, Harry E. Morton has been appointed associate professor of bacteriology and Dale R. Coman assistant professor of pathology.

Society News.—The 1942 Clinical Congress of the American College of Surgeons originally scheduled for October at the Stevens Hotel, Chicago, which was taken over August 1 by the United States Army Air Corps, will be held in Cleveland with headquarters at the Cleveland Public Auditorium, from November 17 to 29, according to an announcement from the college headquarters in Chicago. The twenty-fifth annual Hospital Standardization Conference sponsored by the American College of Surgeons will be held simultaneously.

DEATHS

Deaths.—Adrien Loir, Pasteur's nephew and assistant in 1882 to 1888, has died at the age of 80. Dr. Loir helped to establish Pasteur institutes in St. Petersburg (1886), Sydney (1889) and Tunis (1893). He was director of the institute in Tunis for nine years and medical officer of health of Havre from 1909 to 1939.

Book Reviews

Atlas of Ophthalmic Pathology. Prepared at the Army Medical Museum, Office of the Surgeon General of the United States Army, from material in the Registry of Ophthalmic Pathology. By Elbert DeCoursey, major in the Medical Corps of the United States Army, former pathologist to the registry, and J. E. Ash, colonel in the Medical Corps of the United States Army, curator; Roy M. Reeve, photographer; Helen Campbell Wilder, microscopist; Lawrence P. Ambrogi, technician. Third edition, revised. Pp. 136. Price $5. Omaha: The American Academy of Ophthalmology and Otolaryngology (1500 Medical Arts Bldg.), 1942.

Atlas of Dental and Oral Pathology. Prepared at the Army Medical Museum, Office of the Surgeon General of the United States Army, from material in the Registry of Dental and Oral Pathology. By Joseph L. Bernier, major in the Dental Corps of the United States Army, pathologist to the registry; James B. Mann, colonel in the Dental Corps of the United States Army, former pathologist to the registry; J. E. Ash, colonel in the Medical Corps of the United States Army, curator. Second edition, revised. Pp. 178. Price $5. Chicago: The American Dental Association (212 East Superior Street), 1942.

Atlas of Otolaryngic Pathology. Prepared at the Army Medical Museum, Office of the Surgeon General of the United States Army, largely from material in the Registry of Otolaryngic Pathology. By J. E. Ash, colonel in the Medical Corps of the United States Army, curator, with the assistance of J. L. Bernier, captain, D. C., assistant pathologist; Roy M. Reeve, photographer. Third edition, revised. Pp. 173. Price $5. Omaha: The American Academy of Ophthalmology and Otolaryngology (1500 Medical Arts Bldg.), 1942.

These three atlases, revised and enlarged in new editions, illustrate microscopic pathology, each in its field. They are the outcome of educational programs carried out by the associations concerned. In connection with the programs, registries of specimens from many sources were established in the Army Medical Museum, to the curator of which Col. J. E. Ash, special credit is due for the atlases. The subjects are presented according to the case method. The microscopic sections and the photomicrographs were made at the museum. The descriptions of the microscopic appearances are good and the clinical notes helpful. The unqualified comment that squamous carcinoma of the larynx is resistant to radiation effect is misleading, because the resistance depends not on the structure of the carcinoma but on the extent of its invasion of the laryngeal tissues. The printing and reproduction of the photographs by the planograph process are also pleasing, and the loose leaf binding meets the requirements fully. The arrangement of the photographs is practical and the fields ample. The number of pages listed in connection with the title of each atlas is only half of the actual number of pages, the other half being occupied by the photographs. These are all in black and white. In only a very few instances is the lettering of the photographs rather indistinct or absent. The appeal throughout is to the clinician ("the busy clinician") who wants to advance his understanding of the clinical conditions with which he is dealing, and the atlases will be excellent guides in the study of the microscopic alterations in question. Pathologists also will be interested in the atlases, especially perhaps in the one on ophthalmic pathology. All participants in the production of the atlases are congratulated on the admirable results of well conducted cooperation by clinicians and pathologists and private and governmental agencies.

Books Received

THIRTY-NINTH ANNUAL REPORT, 1941-1942, OF THE IMPERIAL CANCER RESEARCH FUND. Pp. 25. London: Royal College of Surgeons, 1942.

THE DYNAMIC STATE OF BODY CONSTITUENTS. Rudolf Schoenheimer, M.D., late associate professor of biological chemistry, Columbia University. Pp. 78. Price $1.75. Cambridge: Harvard University Press, 1942.

BLOOD GROUPING TECHNIC: A MANUAL FOR CLINICIANS, SEROLOGISTS, ANTHROPOLOGISTS AND STUDENTS OF LEGAL AND MILITARY MEDICINE. Fritz Schiff, M.D., late chief of the Department of Bacteriology, Beth Israel Hospital, New York, and William C. Boyd, Ph.D., associate professor of biochemistry, Boston University School of Medicine; associate member, Evans Memorial, Massachusetts Memorial Hospitals, Boston, with a foreword by Karl Landsteiner, Rockefeller Institute for Medical Research. Pp. 248 with 45 illustrations. Price $5. New York: Interscience Publishers, 1942.

PRIVY COUNCIL, MEDICAL RESEARCH COUNCIL, CHRONIC PULMONARY DISEASE IN SOUTH WALES COALMINERS. I. MEDICAL STUDIES. A. Report by the Committee on Industrial Pulmonary Disease. B. Medical Survey, by P. D'Arcy Hart and E. A. Aslett, with contributions by D. Hicks and R. Yates. C. Pathological Report, by T. H. Belt with assistance from A. A. Ferris. Price $2.90. Pp. 222. London: His Majestry's Stationery Office, 1942.

AMBASSADORS IN WHITE, THE STORY OF AMERICAN TROPICAL MEDICINE. Charles Morrow Wilson. Pp. 372 with illustrations. Price $3.50. New York: Henry Holt & Company, 1942.